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α-Amylase Assays—Calculation of Results

Sirs,

Duffus states[4] that if his method for calculating α-amylase activities by the starch-iodine colour (SIC) method[2,5] is employed using only data from the early stages of the assay reaction, then it will generate correct results. We have pointed out that his method is faulty[5] without giving clear reasons for this statement. We wish to rectify this situation.

Duffus cites results (Table I in his paper)[3] in support of his view. However, the initial linear phase of a plot of log corrected spectrophotometer reading *vs* time is approximately linear[5] only in the range E_{1cm} 565 nm, 1·10–0·70, and as the colour intensity data from the cited table extend from 0·791 to 0·355 then by Duffus' own criterion[4] his calculation should fail. An explanation for the constancy of the k values in the table[3] might be that they refer to calculations made on measurements using the older,[1] and unsatisfactory[2,5] IDC method. This possibility is strengthened by the low 'zero-time colour value' reported in the table (0·791), and by Duffus' failure to distinguish between the two fundamentally different assay procedures.[1,2] Indeed his references to these methods are cited the wrong way round.[3]

Calculations,[3] or more simply plots of log corrected spectrophotometer readings *vs* time, could be used in the inconveniently narrow colour range E_{cm} 565 nm = 1·10–0·70 (when the plot is approximately linear) to assess enzyme activities using the SIC method[2], *only* if 't' were redefined with reference to a colour change occurring within this range. The reason is as follows: α-Amylase activity (SIC) is defined[5] as 100/t, where t is the time (min) for the starch in a standard digest to be degraded to an extent such that the colour it gives with iodine falls from 1·000 to 0·500. The plot of log corrected spectrophotometer reading *vs* time departs significantly from linearity at values below E_{1cm} 565 nm = 0·70, and this deviation is large at E_{1cm} 565 nm = 0·50. Duffus' caculation relies on the 'rate constant', k, indeed being a constant over the range 1·00–0·500, and bearing a fixed relationship to t, $\left(k = \frac{-2{\cdot}303 \log 0{\cdot}5}{t}\right)$. The curvature of the graph demonstrates that k is not constant over the important range, so it cannot be used in a meaningful manner in the way described.

In Duffus' example[3] starch iodine colour readings x_0, x_1, x_2 and x_3 are obtained for samples at time t_0, t_1, t_2 and t_3 respectively. Three k values are then calculated from $k = (\ln x_0 - \ln x_n)/(t_n - t_0)$, where n = 1, 2 and 3 in turn and the final value of k is taken as the mean of these three. According to Duffus, any lack of linearity of the implied plot of ln x against t should be instantly detectable as a large change in k, a view he believes to be so obvious as to be hardly worth mentioning. This might be arguable if his calculations were based on a graphical method and there were ample data; it is neither true nor obvious, however, for the tabular method described,[3] using the sparse data which it was implied were sufficient. To detect a significant trend in the three k values, one would need an independent measure of the precision and it would help if the k values were independently measured. However, there is[3] no suggestion of how to estimate the precision, and the k values are certainly not independent. The derivation of each of the three k values[3] involves the reading x_0 at time t_0. The final average is derived from all four observations weighted in an arbitrary and peculiar way, as can be seen from the following, in which $\bar{k}$ is the averaged constant and it is assumed (as in Duffus' example[3]) that the t values are equally spaced.

$$\bar{k} = \tfrac{1}{3}\left(\frac{\ln x_0 - \ln x_1}{t_1 - t_0} + \frac{\ln x_0 - \ln x_2}{t_2 - t_0} + \frac{\ln x_0 - \ln x_3}{t_3 - t_0}\right)$$

$$= \frac{11\ln x_0 - 6\ln x_1 - 3\ln x_2 - 2\ln x_3}{18(t_1 - t_0)}$$

One characteristic of this formula is that it attaches little weight to x_3 and consequently decreases the likelihood of detecting deviations from linearity.

D. E. Briggs, A. J. Cornish-Bowden & M. T. Smith (1980) "The calculation of results of α-amylase assays" *J. Inst. Brew.* **86**, 162–163

Duffus also states[4] that 'initial velocity is what counts in enzyme assay', a view that contrasts with his proposed method of calculation, based on a determination of a first-order reaction constant for a non-linear reaction.[3] If an assay is being used to derive data for particular kinetic studies then initial reaction rates may well be needed. However, this is not necessarily true when enzyme activity is being used to assess the quantity of an enzyme present in an extract, and it is not true when the SIC method[2,5] is being employed. The reciprocal of the time taken for any fixed proportion of starch degradation to occur (e.g. between 80% and 40%, or whatever) could be used as a measure of enzyme activity as well as the change specified in our calculation.[2,5] Of course, any valid measure of enzyme activity will have a fixed relationship to the initial reaction rate determined under fixed conditions.[5] However, the only situation in which they are the same is when the reaction being followed is linear with time. In view of these considerations we adhere to our original statement,[5] that Duffus' method of calculation[3] is unsound.

Yours sincerely,
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References

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