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The calculation of results of α-amylase assays

Sir,

We do not wish to prolong this correspondence, but various points in Duffus' recent letter[1] require clarification.

Firstly, the fact that a principle of calculation is claimed to be applicable to the results of both sound and unsound assay methods is no measure of its strength, or proof of its applicability to either. As one of us is the originator of both the IDC and SIC methods for estimating α-amylase[2,3] it is incumbent upon us to re-assert that the IDC method[2] should not be used, and continuing references to it are unhelpful when a reader might incorrectly infer that this is a reasonable alternative to the SIC method. Quite apart from other fundamental differences, with results obtained with the IDC method[2] the graph of log corrected colorimetric reading *vs* experimental time is approximately linear in the range 3 to 1, then it gently curves so that the log colour value decreases more slowly with incubation time.[2] In contrast with results obtained by the SIC method[3,4] the graph of log corrected spectrophotometer reading, E_{cm}565nm *vs* time consists of two straight lines joined by a curve extending between E_{cm}565nm 0·7 and 0·3.[4] In this second instance the decline in log colour accelerates in the later stages of the reaction. Thus the kinetics of starch degradation are different in the two systems.

Secondly, it may appear from the small diagram published by Smith, Cornish-Bowden & Briggs[4] (Fig. 1) that the initial linear phase extends to beyond an E_{cm}565nm value of 0·7. However, Duffus is wrong in supposing that we 'misquote our own results'.[5] Inspection of the original data shows that departure from linearity does indeed become detectable at values below 0·7. Duffus is also wrong in supposing that the redefinition of SIC units pre-supposes that the 'zero-time corrected value' is always exactly E_{cm}565nm = 1·000. All that the new definition[4] does, in essence, is to allow the exact calculation of enzymic activities, in SIC units, when this desirable initial value is not precisely achieved. In his original paper[6] Duffus incorrectly expressed the results of his calculation in SIC units using data which, as he now accepts,[1] fall far outside the acceptable range of colour values which may be used in this way.[5]

We readily agree[5] that the principle of Duffus' method of calculation,[6] either using the determination of the initial reaction rate constant, or a graphical representation of log corrected spectrophotometer reading against experimental time, can be employed to obtain a valid estimate of α-amylase activity, and in SIC units, provided that certain points are taken into account. Adhering exactly to the experimental condition already outlined[3,4] samples are taken at known times and their 'starch-iodine colours' are measured. The corrections for blanks are applied, and values which fall into the range E_{cm}565nm 1·10–0·70 are used, either graphically or by calculation, to obtain t(extrapolated), the time that would be taken for the value of the corrected reading to decline from 1·000 to 0·500, if the linear portion of the log colour *vs* time graph extended down to 0·500. However, this exceeds t, the real time in which E_{cm}565nm actually declines from 1·000 to 0·500 by about 10%. Since in our hands replicate determination with this method agree within about 2% we regard an error of 10% as substantial,[4,5] although Duffus apparently does not.[1] Therefore:

$$\alpha\text{-Amylase activity (SIC units)} = \frac{100}{t} = \text{approximately } \frac{100}{t(\text{extrapolated})}$$

In each case the units of t and t(extrapolated) are minutes. However, when the starch iodine colour values of samples from an experimental digestion mixture occur over the full useful colour range (E_{cm}565nm 1·10 to a little below 0·10) one must either use the method of calculation we have previously described[3,4] or dilute the enzyme solution and repeat the determination until the values fall within the range E_{cm}565nm 1·10–0·70.

Yours faithfully,

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References

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