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APPENDIX

The Precision of Analysis by Photoelectric Spectrophotometry

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Twyman & Lothian (1933) showed that the percentage error of photoelectric measurements of extinction, E , is at a minimum for $E=0.43$. If $\delta\theta$ =smallest detectable galvanometer deflexion, T =transmission= I_t/I_0 and δT =smallest measurable transmission change, then $\delta T=k\delta\theta$, where k is constant for a given voltage drop across the potentiometer. Since

$$E = \log 1/T, \quad dE = -dT/2.3T = -dT 10^x/2.3,$$

and hence, if δT is small, the smallest measurable extinction change is

$$\delta E = -\frac{10^x}{2.3} \delta T. \quad (1)$$

The precision of extinction measurements therefore decreases with increase of extinction. The proportional error of extinction measurements is given by

$$p = \frac{\delta E}{E} = -\frac{10^x}{2.3E} \delta T, \quad (2)$$

and is a minimum when $E=0.43$.

With the Beckman spectrophotometer at minimum potentiometer sensitivity, we find

$$\delta T = 2 \times 10^{-3}.$$

The corresponding absolute and proportional errors of extinction measurements, calculated from equations (1) and (2), respectively, are plotted against extinction in Fig. 1: if Beer's law is obeyed, these curves show the errors in concentration measurements. At maximum sensitivity, $\delta T=10^{-3}$, and the errors are half as great.

If a spectrophotometer be set to read $E=0$ with an absorbing solution A , the reading with a second solution B is the extinction difference, $\Delta E = E_B - E_A$. Equations (1) and (2) will apply if ΔE is substituted for E , and it follows that, for a given value of δT , a more precise estimation of ΔE will be obtained in this way than by measuring E_A and E_B separately. This principle can be used, for example, to determine accurately the ratio of the optical depths of two cells.

Analysis of reaction and equilibrium mixtures

For an isolated or reversible reaction in which reactant R is transformed directly into a single stable product P , the extinction of the reacting mixture at any wavelength for which R and P are the only absorbing species is given by

$$E_t = \epsilon_R C_t d + \epsilon_P (C_o - C_t) d,$$

Fig. 1. Calculated precision of extinction measurements with a Beckman spectrophotometer at minimum sensitivity; δE = smallest detectable extinction change.

where C_o = initial molar concentration of R , C_t = concentration of R at time t , d = optical depth, and ϵ_P , ϵ_R = molar extinction coefficients of R and P . The fraction y of reactant remaining at time t is

$$y = \frac{C_t}{C_o} = \frac{E_t \sim \epsilon_P C_o d}{(\epsilon_R \sim \epsilon_P) C_o d} = \frac{E_t \sim E_P}{E_R \sim E_P}, \quad (3)$$

where E_R and E_P are the extinctions of solutions of R and P of concentration C_o , measured at the same optical depth and wavelength as E_t . If such solutions are used as calibration fluids, the calculation of y will be independent of the optical depth and the wavelength, and the accuracy of the analysis will depend only upon (i) the precision of the measurements, and (ii) the accuracy with which the reaction mixture and the calibration fluids are made up. For a reversible reaction, equation (3) with $E_t = E_e$ will give the composition of the mixture at equilibrium, and is applicable, for example, to the spectrophotometric determination of pH with indicators and with buffer solutions of extreme pH ($pK_{in} \pm 3$) as calibration fluids.

(i) *Precision of the measurements.* Since extinction differences are obtained most accurately by direct measurement, the spectrophotometer should be set to $E = 0$ with the calibration fluid having the smaller extinction coefficient. The reading with the other calibration fluid will be the extinction change for complete reaction, $\Delta E_{100\%}$. If ΔE_t is the

reading with the reaction mixture, equation (3) becomes:

$$\text{when } \epsilon_R > \epsilon_P \quad y = \frac{\Delta E_t}{\Delta E_{100\%}}, \quad (4a)$$

$$\text{and when } \epsilon_P > \epsilon_R \quad (1-y) = \frac{\Delta E_t}{\Delta E_{100\%}}, \quad (4b)$$

where $(1-y)$ = fractional concentration of product P .

The absolute error in measuring ΔE_t increases with ΔE_t , according to equation (1). For a given value of $\Delta E_{100\%}$, therefore, equations (4a) and (4b) show that the absolute error in y will increase with y if $\epsilon_R > \epsilon_P$, and decrease with increase of y if $\epsilon_P > \epsilon_R$. The first condition will give the smaller variation of the proportional error with y , and is therefore to be preferred for kinetic measurements over the whole course of a first-order reaction, for which $\log y$ is the significant quantity. This is one factor governing the choice of the most suitable wavelength for the analysis.

For given substances R and P , $\Delta E_{100\%}$ is determined by the wavelength, the initial reactant concentration and the optical depth. For the case $\epsilon_R > \epsilon_P$, the absolute error in y , δy , is related to the proportional errors in ΔE_t and $\Delta E_{100\%}$, p_t and $p_{100\%}$ respectively, by

$$\delta y = y \sqrt{(p_t^2 + p_{100\%}^2)}. \quad (5)$$

Since p is minimum when $\Delta E = 0.43$ (Fig. 1), and since $\Delta E_t < \Delta E_{100\%}$, equation (5) shows that δy will decrease with increase of $\Delta E_{100\%}$ up to

$$\Delta E_{100\%} = 0.43,$$

for all values of y . Higher values of $\Delta E_{100\%}$ will give still smaller errors at low values of y , but greater errors at high values of y . Thus, the optimum value of $\Delta E_{100\%}$ varies with y , but is always greater than 0.43.

These considerations are given quantitative expression in Figs. 2 and 3, which show the errors in determining y with a Beckman spectrophotometer at minimum sensitivity, calculated from equation (5) and the data of Fig. 1. The absolute errors in y for three values of $\Delta E_{100\%}$, 0.4, 0.8 and 1.2, are shown in Fig. 2 as a plot of $100\delta y$ against $100y$: the curves show the absolute errors in measuring the percentage composition of equilibrium mixtures or the absolute errors in measuring the concentration of reactant ($\epsilon_R > \epsilon_P$) or product ($\epsilon_P > \epsilon_R$) expressed as a percentage of the initial reactant concentration. It can be seen that for the highest precision, the wavelength and the optical depth should be chosen with regard to the initial reactant concentration to give $\Delta E_{100\%} = 0.4-0.8$, when the composition of any mixture can be determined with a precision of $\pm 1\%$ of the initial reactant concentration.

For kinetic measurements, the proportional errors in y , $\delta y/y$, are of more significance than the absolute errors. The most uniform distribution of proportional errors over the whole course of a reaction is obtained when $\epsilon_R > \epsilon_P$ and $\Delta E_{100\%} = 0.8$.

Fig. 2. Precision of measurements of the composition of equilibrium or reaction mixtures of substances R and P, for various values of $\Delta E_{100\%} = E_R \sim E_P$, the extinction change for complete conversion of R to P.

Fig. 3. Percentage errors of measurements of the fractional concentration of reactant R in a reaction or equilibrium mixture, with the optimum value of the extinction change for complete reaction, $E_R \sim E_P = 0.8$.

These conditions also give the minimum proportional errors over the second half of the reaction; for the first half, the errors are smaller when $\epsilon_P > \epsilon_R$ and $\Delta E_{100\%} = 0.8$. The calculated percentage errors in y as a function of y are shown in Fig. 3 for these two cases, again for the Beckman spectrophotometer at minimum sensitivity; at maximum sensitivity the errors are half as great.

(ii) *Effect of errors in the initial reactant concentration.* The validity of equation (4) requires that the concentration of each calibration fluid be equal to the initial concentration of the reaction mixture. This condition has practical significance for the flow method, in which the initial reactant concentration depends upon the relative delivery rates through the jets. It will be shown that, for a first-order reaction, the effect of an uncertainty in this quantity upon the accuracy of the analysis depends upon the choice of wavelength and is determined by the relation between the extinction coefficients of reactant and product.

Fig. 4. Percentage errors of measurements of the fractional concentration of reactant R in a reacting mixture resulting from a 1% difference between the concentration of the mixture and that of the calibration fluids. Full lines for $\epsilon_R > \epsilon_P$, broken lines for $\epsilon_P > \epsilon_R$; ϵ_R and ϵ_P = molar extinction coefficients of reactant and product, r = ratio of the smaller extinction coefficient to the difference between them.

Let e = proportional error in the initial reactant concentration, defined by $e = (C_0 - C'_0)/C_0$, where C_0 = concentration of the calibration fluids, and C'_0 = initial reactant concentration in the mixture. Since, for a first-order reaction, the fraction of the total reaction completed in a given time is independent of the initial reactant concentration, the extinction of the reaction mixture, E_t , will also suffer a proportional error e at all values of t , i.e. $\delta E_t/E_t = e$.

If the wavelength is such that $\epsilon_R > \epsilon_P$, equation (4a) is applicable, viz.

$$y = \frac{\Delta E_t}{\Delta E_{100\%}} = \frac{E_t - E_P}{E_R - E_P} \quad (6)$$

Differentiating and substituting for y

$$\frac{dy}{y} = \frac{dE_t}{E_t - E_P} = \frac{dE_t}{E_t} \cdot \frac{E_t}{E_t - E_P}$$

Hence the proportional error in y resulting from a small proportional error e in E_t is

$$p_y = eEt/(E_t - E_p),$$

and substituting for E_t from (6)

$$p_y = e \left\{ 1 + \frac{E_p}{y(E_R - E_p)} \right\} = e \left(1 + \frac{r}{y} \right), \quad (7)$$

where $r = E_p/(E_R - E_p) = \epsilon_p/(\epsilon_R - \epsilon_p)$, that is, the ratio of the smaller extinction coefficient to the difference between them. Alternatively, if the wavelength chosen is such that $\epsilon_p > \epsilon_R$, it can be shown in a similar way from equation (4b) that

$$p_y = e \left(1 - \frac{1+r}{y} \right),$$

where r is again the ratio of the smaller extinction coefficient to the difference between them, in this case $\epsilon_R/(\epsilon_p - \epsilon_R)$.

For given substances R and P , r is determined solely by the wavelength. The error in determining y due to a given error in the initial reactant con-

centration therefore depends solely on the wavelength at which the measurements are made, and, in contrast to the precision of the measurements, is independent of the initial reactant concentration and the optical depth. The error increases with r , and is minimum when $r = 0$, that is when a wavelength is used which either the reactant or the product does not absorb. In Fig. 4, $p_y e$ is plotted against y for values of r of 0 and 2: the curves show the percentage error in y resulting from a 1% error in the initial reactant concentration, calculated from equations (7) and (8). It is evident that with high values of r errors in the initial reactant concentration are magnified considerably in the result, and in the flow method the constancy of the relative delivery rates will be critical. As in the case of the precision of the measurements, the condition $\epsilon_R > \epsilon_p$ gives the more uniform distribution of errors over the whole course of the reaction, and the smaller errors over the second half of the reaction, whilst the condition $\epsilon_p > \epsilon_R$ is better for the first half of the reaction.

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Mould 'Glucosaccharase': a Fructosidase

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Bealing & Bacon (1953) have shown that a trans-fructosidase is probably the only enzyme in extracts of *Penicillium spinulosum* that attacks sucrose. Their experimental data did not exclude the possibility that an additional enzyme attacking sucrose was present in extracts of *Aspergillus oryzae* and *A. niger*.

Further study of the extracts of *P. spinulosum* has shown that the transfructosidase is inhibited by glucose, i.e. it possesses a property previously attributed to a hypothetical α -glucosidase ('glucosaccharase': Kuhn, 1923) and accepted as a criterion to distinguish this enzyme from other invertases. The results presented here suggest that the inhibitory action of sugars and alcohols on mould invertase may be explained by their action as acceptors of fructose residues.

Evidence is also presented that the action of mould extracts on β -fructofuranosides other than sucrose, viz. raffinose, stachyose and melezitose, may be explained without assuming the presence of

a glucosaccharase capable of attacking α -glucosidic linkages similar to that found in sucrose.

These observations lead to the conclusion that there is now little reason to presume that mould extracts contain a glucosaccharase: whether or not enzymes of this type do exist remains to be established.

MATERIALS AND METHODS

The organisms, culture media, and method of preparation of extracts have been described previously (Bealing & Bacon, 1953). This procedure was modified in one instance, when a series of Czapek-Dox media was prepared in which the 5% (w/v) sucrose was replaced by raffinose, maltose, glucose, sorbose, fructose or galactose. Each solution was inoculated with spores of *P. spinulosum* (grown on a sucrose medium) and incubated at room temperature (18–24°). Mycelia were harvested at the beginning of active sporulation, after 8 days on the sucrose, raffinose, maltose and glucose media, and after 14 days on the sorbose and fructose media. Material was not obtained from the galactose media because