85. *The Effect of Experimental Errors on Benesi–Hildebrand Plots and on the Inherent Accuracy of the Equation.*

By P. R. HAMMOND.

An examination of the effect of errors in optical density, acceptor concentration, and donor concentration on the ordinates and abscissae in plots of four forms of the Benesi–Hildebrand relation shows that the scatter of points is different for each form of the equation and depends on the association constant of the complex. Also, an algebraic " two-point " analysis is made of the errors in the association constants and extinction coefficients caused by experimental inaccuracies. A " conspiracy " of errors is found, which increases markedly for decreasing association constants, and it is shown that for very weak interactions even though the relation may apply in theory, it becomes unusable in practice. The best experimental conditions for measurement are discussed.

1. *Introduction.*—An earlier study of complex formation between monosubstituted benzoquinones and hexamethylbenzene[1] was made by examining the dependence of the charge-transfer bands[2] on concentrations of the components. This analysis assumed that the acceptor (benzoquinone) and donor (hexamethylbenzene) associated reversibly in solution to form a 1 : 1 complex and that this was responsible for the new absorption. From studies of a series of solutions in which one of the components was in considerable excess ($b \gg \alpha$ was chosen), equations of the form (2a—d) could be applied where α is the constant, acceptor

$$\text{Acceptor} + \text{Donor} \overset{K}{\rightleftharpoons} \text{Complex} \qquad (1)$$

$$\alpha b/D = 1/K\varepsilon + b/\varepsilon \qquad (2a) \qquad\qquad b/D = 1/K\varepsilon\alpha + b/\varepsilon\alpha \qquad (2b)$$

$$\alpha/D = (1/K\varepsilon)(1/b) + 1/\varepsilon \qquad (2c) \qquad\qquad 1/D = (1/K\varepsilon\alpha)(1/b) + 1/\varepsilon\alpha \qquad (2d)$$

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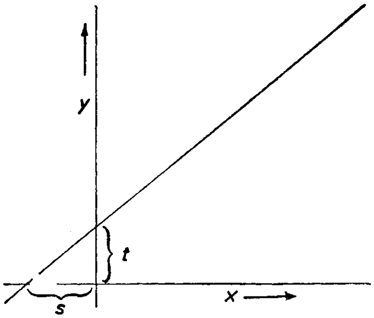
[1] Hammond, preceding paper.

[2] Mulliken, *J. Amer. Chem. Soc.*, 1952, **74**, 811; *J. Phys. Chem.*, 1952, **56**, 801; Mulliken and Person, *Ann. Rev. Phys. Chem.*, 1962, **13**, 107.

concentration, b the varied donor concentration, D the optical density for 1 cm. light-path, corrected for the absorption of the acceptor and usually taken at maximum absorption (if measurements are performed with light path l cm., D/l should be substituted for all values of D in the following discussion), ε the molar absorbance for the complex, and K the association constant for complex formation.

The basis for this relation had been recognised previously[3] and it was first derived by Benesi and Hildebrand from a study of iodine complexes. From plots of $\alpha b/D$ against b (for 2a), b/D against b (for 2b), α/D against $1/b$ (for 2c), or $1/D$ against $1/b$ (for 2d), values of K and ε could be estimated from the intercepts or slopes of the graphs if straight lines were obtained (Figure). The experimental conditions were in fact best represented by means of the equations, and scatter about linear plots was ascribed to experimental errors in measuring optical density, acceptor concentration, and donor concentration. Equations (2a) and (2b) in particular, although they afforded satisfactory plots for the stronger acceptors (cyano- and nitro-benzoquinone), gave marked scatter for systems with low values of K. Other studies on systems which exhibit weak interactions have shown similar scatter in their plots using equations (2a) and (2b).



Plot of the Benesi–Hildebrand equation.

	x	s	y	t
Plot A	b	$1/K$	$\alpha b/D$	$1/K\varepsilon$
Plot B	b	$1/K$	b/D	$1/K\varepsilon\alpha$
Plot C	$1/b$	K	α/D	$1/\varepsilon$
Plot D	$1/b$	K	$1/D$	$1/\varepsilon\alpha$

The practical test of the Benesi–Hildebrand equation and therefore of any theory that is used in deriving it, is a linear plot for equation (2). Hence a study of the effect of errors in measurement on these plots will aid in assessing experimental information and in examining departures from the relation. This is examined in Section 2 for the benzoquinone–hexamethylbenzene study. It is then generalised for other acceptor–donor interactions and the conclusions are set forth in equations (5a—d).

In Section 3 the effect of experimental errors on the values of K and ε estimated from two points, α,b,D and α,b_j,D_j, is considered, and equations (10) and (11) are derived. In Section 4 the results are discussed, especially with regard to optimum conditions of measurement.

The analysis assumes the relation does accurately represent the experimental conditions, an assumption that is certainly justified for strong interactions. It is not concerned with systematic errors arising from the approximations[4] used in deriving equations (2) or in the neglect of activity coefficients.[5] Neither is this article concerned with the physical meaning[6] of the association constants and extinction coefficients obtained from equations (2); rather, K and ε are considered to have discrete values, perhaps unique to a spectrophotometric study, which can be used in their own right as a basis for experimental comparisons.

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[3] Benesi and Hildebrand, *J. Amer. Chem. Soc.*, 1949, **71**, 2703; 1948, **70**, 2832; Hammick and Young, *J.*, 1936, 1463.

[4] Tamres, *J. Phys. Chem.*, 1961, **65**, 654.

[5] Scott, *Rev. Trav. chim.*, 1956, **75**, 787.

[6] Orgel and Mulliken, *J. Amer. Chem. Soc.*, 1957, **79**, 4839; Mulliken, *Rec. Trav. chim.*, 1956, **75**, 845; Hayman, *Chem. Phys.*, 1962, **37**, 2290.

2. *Scatter in Plots.*—If equation (2) is expressed in the form

$$y = mx + c, \qquad (3)$$

then changes in y (δy) and x (δx), arising from small changes $\delta\alpha$, δb, and δD, can be considered.

A graphical plot is most suitably arranged to fill the space of a graph paper. Hence, for comparing a series of acceptor–donor systems where differing values of K and ε apply, the errors in the plots are expressed as a fraction of the total range of y (Δy) and of x (Δx).

A series of measurements was made with donor concentrations, $b_1, b_2 \ldots\ldots b_n$, common to all the benzoquinones. However, the equations apply if the range of concentrations is unique for each acceptor and if measurements are performed with one acceptor and a number of donors.

Also,

$$\delta y = \left(\frac{\partial y}{\partial \alpha}\right)_{b,D}\delta\alpha + \left(\frac{\partial y}{\partial b}\right)_{\alpha,D}\delta b + \left(\frac{\partial y}{\partial D}\right)_{\alpha,b}\delta D \qquad (4)$$

Hence, for equation (2a),

$$y = \alpha b/D,\ x = b,\ \Delta x = (b_n - b_1),\ \Delta y = (b_n - b_1)/\varepsilon$$

$$\left|\frac{\delta y}{\Delta y}\right| \leqslant \frac{(K^{-1} + b)}{(b_n - b_1)}\left(\left|\frac{\delta\alpha}{\alpha}\right| + \left|\frac{\delta b}{b}\right| + \left|\frac{\delta D}{D}\right|\right);\ \left|\frac{\delta x}{\Delta x}\right| = \left|\frac{\delta b}{(b_n - b_1)}\right| \qquad (5a)$$

For equation (2b),

$$y = b/D,\ x = b,\ \Delta x = (b_n - b_1),\ \Delta y = (b_n - b_1)/\varepsilon\alpha$$

$$\left|\frac{\delta y}{\Delta y}\right| \leqslant \frac{(K^{-1} + b)}{(b_n - b_1)}\left(\left|\frac{\delta b}{b}\right| + \left|\frac{\delta D}{D}\right|\right);\ \left|\frac{\delta x}{\Delta x}\right| = \left|\frac{\delta b}{(b_n - b_1)}\right| \qquad (5b)$$

For equation (2c),

$$y = \alpha/D,\ x = 1/b,\ \Delta x = \left(\frac{1}{b_1} - \frac{1}{b_n}\right),\ \Delta y = \frac{1}{K\varepsilon}\left(\frac{1}{b_1} - \frac{1}{b_n}\right)$$

$$\left|\frac{\delta y}{\Delta y}\right| \leqslant (b^{-1} + K)\left(\frac{1}{b_1} - \frac{1}{b_n}\right)^{-1}\left(\left|\frac{\delta\alpha}{\alpha}\right| + \left|\frac{\delta D}{D}\right|\right);\ \left|\frac{\delta x}{\Delta x}\right| = \frac{1}{b}\left(\frac{1}{b_1} - \frac{1}{b_n}\right)^{-1}\left|\frac{\delta b}{b}\right| \qquad (5c)$$

For equation (2d),

$$y = 1/D,\ x = 1/b,\ \Delta x = \left(\frac{1}{b_1} - \frac{1}{b_n}\right),\ \Delta y = \frac{1}{K\alpha\varepsilon}\left(\frac{1}{b_1} - \frac{1}{b_n}\right)$$

$$\left|\frac{\delta y}{\Delta y}\right| = (b^{-1} + K)\left(\frac{1}{b_1} - \frac{1}{b_n}\right)^{-1}\left|\frac{\delta D}{D}\right|;\ \left|\frac{\delta x}{\Delta x}\right| = \frac{1}{b}\left(\frac{1}{b_1} - \frac{1}{b_n}\right)^{-1}\left|\frac{\delta b}{b}\right| \qquad (5d)$$

Equations (5a—d) express the fractional errors in the ordinates and abscissae for each form of the Benesi–Hildebrand equations, (2a—d), in terms of fractional errors in the experimental quantities α, b, and D. In the remainder of this section the major sources of error for each form of the plot are compared by considering the magnitude of the multipliers for the ordinates in equations (5a—d).

For equation (2a) it can be seen that uncertainties in α, b, and D all contribute to errors in the ordinate. Three extreme cases can be considered. (i) A series of studies are performed with donor concentrations ($b_1 \ldots\ldots b_n$) common to all measurements with one * or more † acceptors, and concentrations α are suitably chosen to give measurable optical densities which lie within a preselected range ($D_1 \ldots\ldots D_n$). $\delta y/\Delta y$ changes from a small, constant ‡ error at large K to a rapidly increasing error for decreasing values of K ($K < 1$). (ii) Concentrations α for one or more acceptors are kept constant for a series of measurements and donor concentration ranges ($b_1 \ldots\ldots b_n$) are suitably chosen to give measurable optical densities within a preselected

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* This applies to an examination of one acceptor with concentration ranges ($b_1 \ldots\ldots b_n$) common to a number of donors.

† This applies to an examination of a number of acceptors with a concentration range ($b_1 \ldots\ldots b_n$) of one donor.

range $(D_1 \ldots\ldots D_n)$. Variations in $\delta y/\Delta y$ now depend * primarily on ε. If ε is constant for the series, the fractional error does not depend on the strength of the interaction. However it has been predicted, and in many cases found,[6] that ε rises rapidly for small values of K, so again increased scatter in the plots may occur. (iii) α and the range $(b_1 \ldots\ldots b_n)$ are preselected for a series of studies, and the ranges $(D_1 \ldots\ldots D_n)$ are adjusted by choice of path-length to give measurable readings on the instrument. The reasoning applied to case (i) applies here and rapidly increasing scatter occurs at decreasing values of K.

Between the alternative (i), (ii), and (iii) a range of experimental conditions can be chosen and the most general series of measurements will involve changes in α, in the concentration ranges $(b_1 \ldots\ldots b_n)$ and in the optical-density ranges $(D_1 \ldots\ldots D_n)$. Case (ii) is difficult to achieve in practice, particularly for low values of K, where there is a limit to the concentration b (about 0·3M), consistent with $(b \gg \alpha)$.

The conclusions for cases (i) and (iii) are therefore the most relevant for *all* plots of the Benesi–Hildebrand relation using equation (2a), namely for decreasing values of K $(K < 1)$, a marked increase in scatter occurs for the same errors in the experimental quantities α, b, and D. This also applies for the experimental conditions $(\alpha \gg b)$ by merely interchanging the terms α and b in the above equations and discussion. This conclusion also applies when equation (2b) is used.

Similarly † the multiplier of the ordinates in equations (5c) and (5d) increases for increasing values of K, and is small only when $(K < 1)$. A summary of these conclusions is set out in Section 4.

3. *Inherent Accuracy of the Relation from an Algebraic "Two-point" Analysis.*—In this section, changes in K (δK) and ε $(\delta\varepsilon)$ arising from experimental errors in α, b, and D are considered. Again the changes are expressed as fractions, $\delta K/K$ and $\delta\varepsilon/\varepsilon$.

For any particular complex, measurements are made on b and D as soon as α has been selected and gives measurable optical densities $(D_1 \ldots\ldots D_n)$ for donor concentrations $(b_1 \ldots\ldots b_n)$.

For this analysis the values of K and ε are derived from two experimental points (α,b,D) and (α,b_j,D_j) where

$$\alpha\varepsilon/D_j = 1/Kb_j + 1 \tag{6}$$

From equations (2) and (6),

$$K = (b_jD - bD_j)/bb_j(D_j - D) \tag{7}$$

$$\alpha\varepsilon = DD_j(b_j - b)/(b_jD - bD_j) \tag{8}$$

$$\delta K = (\partial K/\partial b)\delta b + (\partial K/\partial b_j)\delta b_j + (\partial K/\partial D)\delta D + (\partial K/\partial D_j)\delta D_j \tag{9}$$

Hence errors in K and ε are expressed in equations (10) and (11), which are in forms suitable for conditions (i) and (ii) below.

$$\left|\frac{\delta K}{K}\right| \leqslant \frac{(K^{-1} + b_j)}{|b_j - b|}\left|\frac{\delta b}{b}\right| + \frac{(K^{-1} + b)}{|b_j - b|}\left|\frac{\delta b_j}{b_j}\right| + \frac{(1 + Kb)(1 + Kb_j)}{K|b_j - b|}\left(\left|\frac{\delta D}{D}\right| + \left|\frac{\delta D_j}{D_j}\right|\right) \tag{10a}$$

or

$$\left|\frac{\delta K}{K}\right| \leqslant \frac{(\alpha\varepsilon - D)}{|D_j - D|}\left|\frac{\delta b}{b}\right| + \frac{(\alpha\varepsilon - D_j)}{|D_j - D|}\left|\frac{\delta b_j}{b_j}\right| + \frac{\alpha\varepsilon}{|D_j - D|}\left(\left|\frac{\delta D}{D}\right| + \left|\frac{\delta D_j}{D_j}\right|\right), \tag{10b}$$

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* This can be seen by expanding the multiplier of the ordinate of (5a) to:

$$1/K(b_n - b_1) + b/(b_n - b_1) = [(\alpha\varepsilon - D_n)(\alpha\varepsilon - D_1)]/[(\alpha\varepsilon - D)(D_n - D_1)]$$

Case (i). The term $b/(b_n - b_1)$ is common to all values and only the first term of the left-hand side of the equation involves K. Case (ii). The right-hand side of the equation is large for large values of ε.

† This can be seen by expanding the multiplier of ordinates (5c) or (5d)

$$K/(1/b_1 - 1/b_n) + (1/b)/(1/b_1 - 1/b_n) = (1/D)/(1/D_1 - 1/D_n)$$

Case (i). The term $(1/b)/(1/b_1 - 1/b_n)$ is common to all values and only the first term of the left-hand side of the equation involves K. Case (ii). The right-hand side of the equation is common to all values and does not involve variations in K or ε.

and $$\left|\frac{\delta\varepsilon}{\varepsilon}\right| \leqslant \frac{1}{K|b_j - b|}\left(\left|\frac{\delta b}{b}\right| + \left|\frac{\delta b_j}{b_j}\right|\right) + \frac{(K^{-1} + b)}{|b_j - b|}\left|\frac{\delta D}{D}\right| + \frac{(K^{-1} + b_j)}{|b_j - b|}\left|\frac{\delta D_j}{D_j}\right| + \left|\frac{\delta\alpha}{\alpha}\right| \quad (11a)$$

or

$$\left|\frac{\delta\varepsilon}{\varepsilon}\right| \leqslant \frac{(\alpha\varepsilon - D_j)(\alpha\varepsilon - D)}{\alpha\varepsilon|D_j - D|}\left(\left|\frac{\delta b}{b}\right| + \left|\frac{\delta b_j}{b_j}\right|\right) + \frac{(\alpha\varepsilon - D_j)}{|D_j - D|}\left|\frac{\delta D}{D}\right| + \frac{(\alpha\varepsilon - D)}{|D_j - D|}\left|\frac{\delta D_j}{D_j}\right| + \left|\frac{\delta\alpha}{\alpha}\right| \quad (11b)$$

A narrow range of donor concentrations ($|b_j - b|$ and $|D_j - D|$ small) results in the expected large fluctuation in K and ε for an experimental error.

Three extreme experimental situations are considered as before, and, for each of these, two points are selected for determining K and ε. (i) One set of donor concentrations ($b_1 \ldots\ldots b_n$) is chosen common to all measurements with one * or more † acceptors and concentrations α are adjusted to give measurable optical densities, which lie within a preselected range ($D_1 \ldots\ldots D_n$). Hence, errors in equations (10a) and (11a) become very large when K is small, for in all terms involving δb, δb_j, δD, and δD_j, $1/K$ is dominant. In addition, when K is large, equation (10a) shows that K estimations are sensitive to errors in optical density, where the fractional error is proportional to K. The multiplier of errors in optical-density readings has a minimum at

$$bb_j = K^{-2} \quad (12)$$

(ii) Constant values of α are used with suitable donor concentration ranges ($b \ldots\ldots b_n$) to give measurable optical densities within a preselected range ($D_1 \ldots\ldots D_n$). From each of these donor ranges, b and b_j are chosen. The multipliers of all terms involving δb, δb_j, δD, and δD_j in equations (10b) and (11b) are large for large values of ε. If, as before, it is accepted that ε increases markedly for weak interactions [6] ($\varepsilon \longrightarrow \infty$ as $K \longrightarrow 0$), then large relative fluctuations in the estimated K and ε occur for the same experimental errors at small values of K. (iii) α and the range ($b_1 \ldots\ldots b_n$) are preselected for a series of studies and the ranges ($D_1 \ldots\ldots D_n$) are adjusted by choice of path-length. The reasoning applied to case (i) applies here.

Of the alternatives (i), (ii), and (iii), again (ii) is the most difficult to achieve in practice and, for any general experiment, estimation of K and ε is very sensitive to experimental error for weak interactions. Similarly this applies when $\alpha \gg b$.

The usual estimation of K and ε from a number of measurements of b and D is not covered by the present article, although it is probable that the conclusions are relevant to this case. A statistical approach may analyse this situation.

4. *Experimental Implications.*—Section 2 [equations (5a—d)] shows that the effect of experimental errors on plots of the Benesi–Hildebrand relation is to produce a scatter of points about a straight line. This scatter depends on the strength of the interaction between the donor and the acceptor (K), and on whether the relation is expressed as (2a), (2b), (2c), or (2d). The effects of K on the scatter are complementary for the two forms (2a) and (2b), as opposed to (2c) and (2d). Whereas one form produces scatter only at small values of K, the other does so only at large K. In particular, equations (2a) and (2b), although they can be used for displaying information for large K, are most unsuitable when $K < 1$; *e.g.*, for a measurement on a benzoquinone–hexamethylbenzene complex, the values $K = 0{\cdot}3$ l. mole^{-1}, $b = 0{\cdot}2$M, $b_1 = 0{\cdot}05$M, $b_n = 0{\cdot}3$M were typical. For an error in optical-density reading alone (0·01), with an unfortunately low reading of D at 0·20, by equation (5a) or (5b) the error ($\delta y/\Delta y$) in the ordinate is 0·71, in comparison with the total range of experimental measurement represented by unity. Equations (5c) and (5d) give 0·016 for $\delta y/\Delta y$ in this case.

As an equation for general use, (2d) is particularly recommended since scatter in points

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* See footnote * on p. 481. † See footnote † on p. 481.

arises from errors in b and D alone, and values of K and ε can be obtained directly from the graph.*

Errors in all experimental measurements are represented as fractions of the quantity measured. With standard equipment, optical density is considered to be the major source of error where ($\delta D = 0{\cdot}01$) is a typical inaccuracy in zeroing the instrument. For small optical-density values within a series of measurements ($D_1 \ldots\ldots D_n$), $\delta D/D$ can be reduced by increasing the path-length.

Section 3 [eqns. (10) and (11)] shows that estimations of K and ε, from two measurements each of donor concentration and of the associated optical densities for a given α, are dependent on experimental errors. K is affected by errors in b and D, whereas ε is affected by errors in b, D, and α. This effect depends on the strength of the interaction and to some extent on the manner in which the experiment is performed. For the same experimental errors at weak association, the magnitude of the error in K approaches a constant value. Also, the relative errors in K and ε become very sensitive to errors in both b and D, where the error terms are dominated by $1/K$; *e.g.*, the high concentrations $b_j = 0{\cdot}3$M and $b = 0{\cdot}05$M, typical for measurements on the weaker benzoquinones, can be used to estimate K and ε. The Table shows $\delta K/K$ and $\delta\varepsilon/\varepsilon$ [eqns. (10a) and (11a)] for differing values of K, arising from errors in D and D_j alone so that $\delta D/D = \delta D_j/D_j = 0{\cdot}01$.

Errors in the estimation of K and ε caused by experimental errors in optical-density readings.

K (l. mole^{-1})	$\delta K/K$	$\delta\varepsilon/\varepsilon$	K (l. mole^{-1})	$\delta K/K$	$\delta\varepsilon/\varepsilon$
1·00	0·11	0·09	0·10	0·83	0·81
0·30	0·30	0·28	0·01	8·03	8·01
0·20	0·43	0·41			

Certainly for ($K < 0{\cdot}2$) δK and $\delta\varepsilon$ are comparable with K and ε, respectively. Improving the accuracy of measurements merely displaces the point at which this condition is reached to smaller values of K, and even if the Benesi–Hildebrand relation still represents the experimental conditions, K and ε derived from it have little practical use.

Equation (10a) also shows that estimations of K are responsive to errors in D at large values of K. In equation (12), if b and b_j are chosen as the extremes of donor concentrations, the sensitivity of K to errors in optical density is least when $b_1 b_n = K^{-2}$, or, from equation (2), when $\alpha\varepsilon = D_1 + D_n$. For the most general experiment, these are therefore the best conditions for measuring K, but they are difficult to achieve unless $5 < K < \varepsilon/12$.†

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* Consideration of this graph leads to some of the conclusions of Section 3. For example, as $\alpha\varepsilon$ and K are determined directly from the intercepts, errors in α affect ε estimates alone and not those of K.

† An upper limit for b of about 0·3M, and a range of optical densities measured by the instrument between 0·5 and 1·5 are imposed. For large values of K, the smallest concentration α consistent with ($b_1 > 20\alpha$) is achieved by increasing the light-path up to 10 cm.