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A Simplified Method for Spectrophotometric Determination of Equilibrium Constants

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For a simple chemical equilibrium of the type

$$C + L \underset{}{\overset{K_{eq}}{\rightleftharpoons}} C\text{–}L \quad (1)$$

where C is a chromophore, L is a ligand or titrant, and C–L is the 1:1 addition product of the reaction of C and L, spectrophotometric determination of the equilibrium constant, K_{eq}, is possible as long as there is a sufficient difference in absorbance of the two chromophoric species, C and C–L, at some usable wavelength. From Beer's Law, the absorbance of any solution containing an equilibrium mixture of C and C–L, at wavelength λ, A^{λ}, is given by eqn. 2:

$$A^{\lambda} = l\epsilon_{C}^{\lambda}[C]_{eq} + l\epsilon_{C-L}^{\lambda}[C\text{–}L]_{eq} \quad (2)$$

where ϵ_{C}^{λ} and ϵ_{C-L}^{λ} are the molar absorptivities of C and C–L, $[C]_{eq}$ and $[C\text{–}L]_{eq}$ are the equilibrium concentrations of C and C–L, and l is the pathlength in cm, assuming L does not absorb at wavelength λ (see below). By application of eqn. 2, the law of mass action

$$K_{eq} = \frac{[C\text{–}L]_{eq}}{[C]_{eq}[L]_{eq}} \quad (3)$$

and the conservation equation

$$C_T = [C]_{eq} + [C\text{–}L]_{eq} \quad (4)$$

one can readily show that equation 5 applies to the spectrophotometric titration of such simple equilibria:

$$\Delta A^{\lambda} = \frac{\Delta A_{max}^{\lambda}[L]_{eq}}{1/K_{eq} + [L]_{eq}} \quad (5)$$

In this formulation, ΔA^{λ} is the absolute value of the difference in absorbance between solutions containing chromophore and ligand and solutions containing chromophore alone at the same concentration (C_T),

$$\Delta A^{\lambda} = |A^{\lambda} - l\epsilon_{C}^{\lambda}C_T| \quad (6)$$

ΔA_{max}^{λ} is the maximal ΔA^{λ} obtainable at high $[L]_{eq}$,

$$\Delta A_{max}^{\lambda} = C_T|\epsilon_{C-L}^{\lambda} - \epsilon_{C}^{\lambda}|l \quad (7)$$

and $[L]_{eq}$ is the *equilibrium* concentration of titrant. The major problem with such determinations is that under many circumstances $[L]_{eq}$ differs significantly from the added amount of titrant, $[L]_T$, due to consumption of added ligand by formation of product. Except in the case of hydronium ion, whose activity at equilibrium can be directly measured using the glass electrode, direct determination of $[L]_{eq}$ is frequently impossible. This problem is usually dealt with by iterative procedures in which an initial guess of K_{eq} is used in conjunction with eqn. 3 to calculate $[L]_{eq}$ for each value of $[L]_T$. The corrected data is then fit to eqn. 5 to provide a better estimate of K_{eq}. This procedure is repeated until the solution converges to a constant value for K_{eq}. Rigorous statistical treatments of this problem are available [1] but such treatments require access to computers with considerable storage capacity and a not inconsequential amount of programming acumen. A chemically correct, but considerably less statistically rigorous treatment is also available [2] but this method is quite tedious without the aid of a computer. I have found that a much simpler, though somewhat less rigorous, treatment provides satisfactory results in a wide variety of cases (limitations to this method are described below). The method is amenable to graphical solution with the aid of the most rudimentary of pocket calculators (or a slide rule for that matter) or to a statistical determination with the aid of a programmable pocket calculator.

The method is based on calculation of $[L]_{eq}$ *via* eqn. 8.

$$[L]_{eq} = [L]_T - C_T\Delta A^{\lambda}/\Delta A_{max}^{\lambda} \quad (8)$$

The ratio $\Delta A^{\lambda}/\Delta A_{max}^{\lambda}$ represents the fractional conversion of chromophore to product so that $C_T\Delta A^{\lambda}/\Delta A_{max}^{\lambda}$ is the concentration of titrant consumed by formation of product in any particular sample. Hence, as long as ΔA_{max}^{λ} can be determined, application of eqn. 8 allows the calculation of $[L]_{eq}$ at each $[L]_T$ used in the determination. The equilibrium constant can then be obtained by a convenient linearization of eqn. 5:

$$\Delta A^{\lambda} = \Delta A_{max}^{\lambda} - \frac{1}{K_{eq}} \cdot \frac{\Delta A^{\lambda}}{[L]_{eq}} \quad (9)$$

For a graphical solution, ΔA^{λ} is plotted as a function of $\Delta A^{\lambda}/[L]_{eq}$ and the slope is $-1/K_{eq}$. For a statistical evaluation a least squares fit of eqn. 9 may be performed. The appropriate equations are readily available [3]. Many relatively inexpensive pocket calculators contain a '$\Sigma+$' key which allows automatic accumulation of the appropriate sums for use in the least squares equations and some even have a linear regression key which will evaluate ΔA_{max}^{λ} and

$1/K_{eq}$ once the appropriate sums have been accumulated. The statistical uncertainties in ΔA^{λ}_{max} and $1/K_{eq}$ may then be approximated using eqns. 10–12 [4]:

$$\sigma(\Delta A^{\lambda}_{max}) = \sigma \sqrt{\frac{\Sigma\left(\frac{\Delta A^{\lambda}}{[L]_{eq}}\right)_i^2}{N\left[\Sigma\left(\frac{\Delta A^{\lambda}}{[L]_{eq}}\right)_i^2 - \frac{1}{N}\left(\Sigma\left(\frac{\Delta A^{\lambda}}{[L]_{eq}}\right)_i\right)^2\right]}} \quad (10)$$

$$\sigma(1/K_{eq}) = \frac{\sigma}{\sqrt{\Sigma\left(\frac{\Delta A^{\lambda}}{[L]_{eq}}\right)_i^2 - \frac{1}{N}\left(\Sigma\left(\frac{\Delta A^{\lambda}}{[L]_{eq}}\right)_i\right)^2}} \quad (11)$$

where

$$\sigma = \sqrt{\frac{\Sigma(\Delta A^{\lambda})_i^2 - \Delta A^{\lambda}_{max}\Sigma(\Delta A^{\lambda})_i - \frac{1}{K_{eq}}\Sigma\left(\frac{\Delta A^{\lambda}}{[L]_{eq}}\right)_i(\Delta A^{\lambda})_i}{N-2}} \quad (12)$$

The latter calculations are facilitated by the use of a programmable calculator. An estimate for the variance of K_{eq} is obtained from $\sigma(1/K_{eq})$ (eqn. 11) by

$$\sigma(K_{eq}) = K_{eq}^2\sigma(1/K_{eq}) \quad (13)$$

It should be pointed out that eqn. 9 is formally similar to the Ketelaar equation [5] (and its simpler relative the Benesi–Hildebrand equation [6]) in which $[L]_T$ appears in place of $[L]_{eq}$. These equations are derived from similar principles under the *assumption* that an insigificant amount of added titrant is bound in any sample, *i.e.* that $[L]_{eq} = [L]_T$. Unfortunately, these equations are often cited without any indication that they are in fact approximate [7].

As an example of the utility of the present method, a data set for the determination of the equilibrium constant for axial ligand substitution of ethyl(aquo)-cobaloxime [8] by the primary amine dimethoxyethylamine is given in Table I.

$$CH_3CH_2Co(D_2H_2)HOH + RNH_2 \xrightleftharpoons{K_{eq}} CH_3CH_2Co(D_2H_2)H_2NR + HOH \quad (14)$$

Figure 1 shows a plot of eqn. 9 for this data set. The straight line is a visual fit of the data and yields the values $\Delta A^{455}_{max} = 0.829$, $K_{eq} = 451\ M^{-1}$, whereas a least squares fit of the data (performed with the aid of a Hewlett-Packard 55 Calculator) produced the values $\Delta A^{455}_{max} = 0.818 \pm 0.008$, $K_{eq} = 472 \pm 11\ M^{-1}$. Figure 2 shows a plot of the raw data (A^{455} *vs.* $[L]_T$). The solid line has been calculated from the least squares results, $C_T = 6.68 \times 10^{-4}\ M$, and $A_0^{455} = 1.163$ (the absorbance of the chromophore in the absence of added ligand) by use of eqns. 3, 5 and 6.

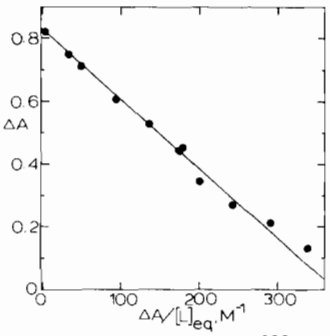

Fig. 1. Plot of eqn. 9, ΔA^{455} *vs.* $\Delta A^{455}/[L]_{eq}$, for the data in the table. The solid line is a visual fit; slope = $-2.22 \times 10^{-3}\ M$, intercept = 0.829, leading to $K_{eq} = 451\ M^{-1}$.

TABLE I. Data for the Spectrophotometric Titration of Ethyl(aquo)cobaloxime with Dimethoxyethylamine.[a]

$[L]_T, M$	ΔA^{455}	ΔA^{455} [b]	$[L]_{eq}$, [c] M	$\frac{\Delta A^{455}}{[L]_{eq}}, M^{-1}$
0	1.162			
0	1.164			
5.00×10^{-4}	1.030	0.133	3.92×10^{-4}	339
9.00×10^{-4}	0.951	0.212	7.28×10^{-4}	291
1.33×10^{-3}	0.893	0.270	1.11×10^{-3}	243
2.00×10^{-3}	0.818	0.345	1.72×10^{-3}	201
2.90×10^{-3}	0.708	0.455	2.53×10^{-3}	180
2.90×10^{-3}	0.720	0.443	2.54×10^{-3}	174
4.33×10^{-3}	0.633	0.530	3.90×10^{-3}	136
7.00×10^{-3}	0.554	0.609	6.51×10^{-3}	93.6
7.00×10^{-3}	0.555	0.608	6.51×10^{-3}	93.5
1.50×10^{-2}	0.450	0.713	1.44×10^{-2}	49.4
2.30×10^{-2}	0.413	0.750	2.24×10^{-2}	33.5
2.30×10^{-2}	0.411	0.752	2.24×10^{-2}	33.6
2.00×10^{-1}	0.343	0.820	1.99×10^{-1}	4.11
5.00×10^{-1}	0.342	0.821	4.99×10^{-1}	1.64
5.00×10^{-1}	0.340	0.823	4.99×10^{-1}	1.65

[a]Conditions: 15.0 ± 0.1 °C, pH 9.33 ± 0.02, ionic strength 1.0 *M* (KCl), λ = 455 nm, $C_T = 6.68 \times 10^{-4}\ M$. Data were collected on a Gilford Model 250 spectrophotometer. [b]Calculated as 1.163 minus the absorbance at 455 nm for each sample. [c]Calculated from eqn. 8, using $\Delta A_{max} = 0.822$.

Practical Aspects and Limitations of the Method

It should first be pointed out that as in all such methods the assumption that the activity coefficients of the reagents (or at least their ratio) is constant throughout the range of concentrations employed is implicit.

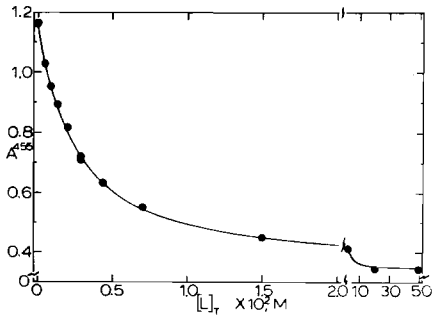

Fig. 2. Plot of the raw data in the table as ΔA^{455} *vs.* $[L]_T$. The solid line has been calculated from the least squares results, $\Delta A^{455}_{max} = 0.818 \pm 0.008$, $K_{eq} = 472 \pm 11\ M^{-1}$, the values of $C_T = 6.68 \times 10^{-4}\ M$ and $A_0 = 1.163$ (absorbance of the chromophore in the absence of added ligand) and eqns. 8 and 9.

Experimental determinations of equilibrium constants are best performed by preparation of a series of samples containing chromophore at a fixed concentration (C_T) and ligand varying from about $0.2/K_{eq}$ to about $100/K_{eq}\ M$. An accurate determination of the absorbance of the chromophore at C_T in the absence of ligand is also required. The chromophore concentration should be adjusted to provide a value of $\Delta A^{\lambda}_{max} > 0.450$ for highest precision work, although ΔA^{λ}_{max} as low as 0.300 can produce satisfactory results if careful measurements are made. In our research we generally try to fit data sets containing 12–15 points and are usually successful at obtaining equilibrium constants with relative uncertainties of < 5%. However, the method can be successfully used with as few as five data points to yield equilibrium constants with relative uncertainties of about 10%.

Unless the spectral properties of the chromophore and product are well known, and a reasonable guess at the equilibrium constant is in hand, it is often a good idea to perform a preliminary experiment on a scanning spectrophotometer using several widely varying concentrations of L. This will provide an estimate of K_{eq} which can be used to calculate appropriate concentrations of L for a precise determination and also allows selection of an appropriate wavelength for the experiment.

The complication of a ligand which absorbs at the measurement wavelength is easily handled by this method. Since the equilibrium concentration of ligand can be calculated for each sample, a determination of the molar absorptivity of the ligand allows a calculation of the amount of absorbance of each sample due solely to the absorbance of free ligand. In such cases the value of ΔA^{λ} for each sample is simply corrected by subtraction of $\epsilon^{\lambda}_{L}[L]_{eq}$, where ϵ^{λ}_{L} is the molar absorptivity of the ligand in the absence of chromophore. The resulting corrected values of ΔA^{λ} may then be used directly in conjunction with eqn. 9 to evaluate the equilibrium constant. Naturally it is desirable to minimize these corrections by working at a wavelength where ϵ^{λ}_{L} is as small as possible.

The principal limitation to the method is the limitation on the magntidue of K_{eq} which occurs to a lesser extent with even the most rigorous of methods. For values of K_{eq} which exceed $1/C_T$, the amount of titrant consumed at the lower end of the titration becomes a large fraction of the added titrant. Experience shows that when the amount of free titrant is less than about 50% of added titrant at the lowest titrant concentration used the method becomes unreliable and should be used with caution. As a practical limit, it will be difficult to obtain reliable values of K_{eq} when K_{eq} exceeds about $4|\epsilon^{\lambda}_{p} - \epsilon^{\lambda}_{c}|$ using one centimeter pathlength cells. The utility of the method for large K_{eq}'s can be considerably extended by use of longer pathlength cells (5 or 10 cm) which allows considerable lowering of C_T and hence decreases the amount of titrant bound in any sample.

The second limitation of the method is due to the necessity of an accurate determination of ΔA^{λ}_{max} for use in eqn. 8. This requires measurement of the absorbance of a sample containing ligand at a concentration of about $100/K_{eq}\ M$. In most cases where such a measurement is impossible it is because of a low value of K_{eq}. Fortunately in these cases the amount of titrant consumed is insignificant with respect to the amount of added titrant and the need for calculation of $[L]_{eq}$ *via* eqn. 8 is obviated (these represent the special cases for which the Ketelaar equation [5] is valid). However, in some cases where correction for bound titrant is required, determination of ΔA^{λ}_{max} may be prevented by limited solubility of L. The method may still be applied in such cases by use of an iterative procedure as is common with other, similar methods [9]. The best available estimate of ΔA^{λ}_{max} is used to fit the data and provide improved estimates of K_{eq} and ΔA^{λ}_{max}. The improved value of ΔA^{λ}_{max} is then used in another round of calculations, *etc.*, until values of desired precision are obtained.

Finally, it must be mentioned that use of the linearized binding function (eqn. 9) causes distortion of the error function such that points at low values of $[L]_{eq}$ are weighted more heavily than those at high $[L]_{eq}$ during the regression analysis. Fortunately the problem is not very severe for the particular linearization used in this method. This problem can be dealt with by doing an appropriately weighted least squares analysis of the fit to eqn. 9 [3]. However, such a calculation requires more programming capacity than is available on most reasonably priced programmable pocket calculators. Extensive experience with the method shows that inclusion of two or three points at ligand concentrations high enough to insure > 90% conversion of chromophore to product is sufficient

to provide the necessary weight to the high end of such titrations. Thus, the sample used to determine ΔA^{λ}_{max} should always be used in the fit to eqn. 9.

References

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