Generalization of the Method of Lang for the Spectrophotometric Determination of the Composition and Stability of Weak Complexes

GUNNAR NORHEIM*

Chemical Institute A, University of Oslo, Blindern, Oslo 3, Norway

A general method for the determination of the stability constant and the extinction coefficient of weak complexes from spectrophotometric data is described. Linear least squares analysis, performed by computer, is used in the calculations. When calculations are made for different trial compositions for one set of data, the correct composition can be found from the standard deviations. The method is tested with theoretical and experimental spectrophotometric data.

Spectrophotometric data are often used for the determination of the composition and the stability constant of different complexes. Lang [1] has described a method for the determination of the stability constant of 1 : 1 complexes. In the present paper, the Lang method is generalized to handle complexes with any composition $m : n$.

THEORY

Considering the equilibrium

$$mA + nB = A_mB_n \tag{1}$$
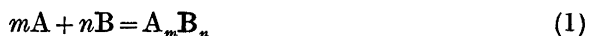
the stability constant is

$$K = \frac{c}{(a - mc)^m (b - nc)^n} \tag{2}$$

where c is the concentration of the complex, and a and b are the initial concentrations of A and B. According to the Beer-Lambert law

$$c = E/\varepsilon d \tag{3}$$

---

* Present address: Institute of Forensic Medicine, University of Oslo, Rikshospitalet, Oslo 1, Norway.

where E is the measured extinction, ε is the molar extinction coefficient, and d is the cell length. A combination of eqns. (2) and (3) gives

$$\left[a - m\,\frac{E}{\varepsilon d}\right]^m \left[b - n\,\frac{E}{\varepsilon d}\right]^n = \frac{E}{\varepsilon d K} \tag{4}$$

This can be rearranged, giving

$$\frac{a^m b^n}{E} = \left[\binom{n}{1}a^m b^{n-1}n - \binom{n}{2}a^m b^{n-2}n^2\frac{E}{\varepsilon d} + \cdots\right.$$

$$- (-1)^n a^m n^n \left(\frac{E}{\varepsilon d}\right)^{n-1} + \binom{m}{1}a^{m-1}b^n m$$

$$- \binom{m}{1}\binom{n}{1}a^{m-1}b^{n-1}mn\,\frac{E}{\varepsilon d} + \cdots$$

$$\left. - (-1)^{m+n}m^m n^n \left(\frac{E}{\varepsilon d}\right)^{m+n-1}\right]\frac{1}{\varepsilon d} + \frac{1}{\varepsilon d K} \tag{5}$$

where $\binom{m}{1}$, $\binom{n}{1}$, etc., are the binomial coefficients. According to eqn. (5), a straight line will result if $a^m b^n/E$ is represented graphically as a function of the expression in the square brackets, the slope of the line being $1/\varepsilon d$.

Eqn. (5) can be used as a basis for a graphical method for the determination of K. If ε is known, eqn. (5) contains only known characters except for K, and K can be calculated from the intercept on the ordinate axis. However, in most cases, ε is unknown, and the calculation of the abscissa value in the square brackets must be started with an initial value ε_1. From the line that can be drawn, a new value ε_2 is calculated from the slope. This value ε_2 is then used to calculate new values of the expression in the square brackets. This gives ε_3, and so on. When the difference between two values $\Delta\varepsilon$ calculated after each other is less than a certain limit, K can be calculated from the intercept on the ordinate axis. These calculations are easily made with a computer, using linear least squares analysis. For a given set of data, the standard deviations, σ_K and σ_ε in K and ε, respectively, can also be calculated.

By this method it is possible to determine the composition of the complex from the calculated standard deviations in K and ε. K and ε are calculated for different trial compositions from the same set of data, the correct composition giving the smallest standard deviation. This is demonstrated later.

THEORETICAL DATA

The method was tested theoretically with data from calculated mole ratio curves. The mole ratio curves were calculated from the law of mass action. Eqn. (2) was rearranged to

$$(a - mc)^m (b - nc)^n - \frac{c}{K} = 0 \tag{6}$$

When $x = a/b$ and $y = c/b$, this becomes

$$(x - my)^m (1 - ny)^n - \frac{y}{Kb^{m+n-1}} = 0 \tag{7}$$

In the mole ratio method, b is kept constant, and x is the mole ratio between A and B. y is proportional to the extinction: $y = E/\varepsilon db$. For convenience, εdb was chosen equal to unity in the calculations. m, n and K were chosen for each calculated mole ratio curve. At infinite excess of A, y will be equal to $y_0 = 1/n$. The value of W

$$W = \frac{1}{Kb^{m+n-1}} \tag{8}$$

defines the mole ratio curve, and is a measure of the deviation from the hypothetical curve for the infinite stable complex.*

The optimum conditions for the determination of the stability constant of weak complexes have been calculated previously,[2] and an optimum factor N_{op} has been defined. The theoretical data were chosen according to these calculations with $N_{op} = 2.0$, when x varied less than by a factor of ten, and $N_{op} < 2.0$, when this still gave a ten times variation in x. Each series consisted of twelve sets of data, and the x-values were equally spaced.

From the theoretical mole ratio curve obtained in this way, K and ε and their standard deviations σ_K and σ_ε were determined by the present method for different trial compositions as shown below.

THE STANDARD DEVIATION

In eqn. (5), the greatest part of an experimental uncertainty comes from the measured extinction E. Therefore the uncertainty in a and b are set equal to zero. In the calculated value of the square brackets, the expressions where E occurs give only small contributions to the total value. Therefore it is assumed that all the uncertainty comes from the ordinate. Using the ordinary method of linear least squares to fit the line $y = ax + b$, the standard deviations can be calculated.[3]

CALCULATIONS

All the calculations were made with a special computer program. For a given set of data where the composition and stability constant had been chosen, K, ε, σ_K and σ_ε were calculated as described above. The calculations were made for the following compositions: $1:1$, $1:2$, $1:3$, $1:4$, $2:2$, $2:1$, $3:1$, and $4:1$, and for the following values of W: $W = 1.0 \times 10^p$, $p = 4, 3, 2, 1, 0$, and -1. The initial concentration of B (the component kept constant) was chosen to be $b = 1.0 \times 10^{-4}$. For the correct composition this should give $K = 1.0 \times 10^q$, where $q = 4(m + n - 1) - p$.

---

* These calculations were originally proposed by K. S. Klausen.

Table 1. Calculated values for K, σ_K and ε for different trial compositions. The correct composition is chosen to be $1:1$. $W=1.0\times10^p$ and $p=4-q$.

Trial composition $m:n$	Obtained conditional constant K	Standard deviations (%)			Extinction coefficient	
		$\sigma_K(p=4,3,2,1)$	$\sigma_K(p=0)$	$\sigma_K(p=-1)$	$\varepsilon\times10^{-4}(p=4,3,2,1,0)$	$\varepsilon\times10^{-4}(p=-1)$
1 : 1	1.00×10^q	0.0	0.0	0.0	1.00	1.00
1 : 2		2.3 ± 0.1	5.8	11.5	2.9 ± 0.1	3.5
1 : 3		3.0 ± 0.1	7.0	11.0	5.7 ± 0.1	7.2
1 : 4		3.4 ± 0.1	7.6	10.9	9.4 ± 0.2	12.1
2 : 2		8.1 ± 0.3	9.4	9.5	1.9 ± 0.1	1.9
2 : 1		>14.5	43.1	51.9	0.80 ± 0.05	1.2
3 : 1		>25.6	>100	a	0.80 ± 0.05	a
4 : 1		>33.6	38.6	41.8	0.80 ± 0.05	2.2

a No convergence.

Table 1 gives the result of the calculations when the correct composition is $1:1$. When the tabulated values are of the form $g\pm h$, g is the mean of the highest and lowest value, and h is the half of the difference between these two numbers. It was found that the calculated values for K and σ_ε gave little information. The order of magnitude of K did not say much about the value being reasonable, and σ_ε did not vary much for the different calculations. Therefore only σ_K and ε are tabulated. σ_K's are calculated in per cent.*

In some special cases, the computer program made was not able to calculate K and ε. These cases are marked *no convergence* in the tables, and these calculations are omitted in the tabulated values. In most cases, the value for the extinction coefficient, calculated from the slope, converges rapidly to its final value, and $\Delta\varepsilon^2=1.0$ used. However, for the compositions $3:1$ and $4:1$, and $W\leq1.0$, this gave a wrong value for K. For $W=1.0$, and for $3:1$ and $W=0.1$, it was used $\Delta\varepsilon^2=0.01$, and for $4:1$ and $W=0.1$, $\Delta\varepsilon^2=0.0001$ was used.

For the compositions $1:1$, $1:2$, $1:3$, and $1:4$ for $W\geq1.0$, and for $1:3$ and $1:4$ for $W=0.1$, $N_{op}<2.0$ was used for the theoretical data. If $N_{op}=2.0$ was used, and x varied more than by a factor of ten, the standard deviations in K became somewhat greater. This gave a better indication of the correct composition, but it will not always be convenient in practical work.

For the theoretical data, the number of measurements in each series was not critical. Calculations were made with theoretical data, covering the same concentration range as those used in Table 1 and in the tables not printed here, but with only six measurements in each series. This gave values for σ_K and ε, which were equal to (or only slightly different from) those tabulated. In practical work, however, where the different quantities are encumbered with experimental uncertainties, the number of measurements should not be too small. An experimental series with ten or more measurements covering a suitable concentration range should give a reliable result.

---

* On request, tables with the same calculations as in Table 1, but where the correct composition is $1:2$, $1:3$, $1:4$, $2:2$, $2:1$, $3:1$ or $4:1$, can be obtained from the author.

THE RELIABILITY OF THE METHOD

If the initial concentrations or the measured extinctions are encumbered with systematic errors, this can cause more or less trouble. If all extinctions in a series are increased or decreased with 5 %, this would give an error of $\pm 5\%$ in ε, but no error in K. An error of $\pm 2\%$ in a or b would give errors in both K and ε. This is demonstrated in Tables 2 and 3.

Tables 2 and 3 show that the errors in K and ε are dependent on the compositions. It is also seen that the errors in most cases increase with increasing stability.

Table 2. Deviation in per cent from correct value of K and ε, when an error of $\pm 2.0\%$ is induced into the initial concentrations of the component varied in excess. $W = 1.0 \times 10^p$.

	$a' = a \times 1.02$			$a' = a \times 0.98$		
$m:n$	δ_K $p = 4, 3, 2, 1, 0$	δ_K $p = -1$	δ_ε $p = 4, 3, 2, 1, 0, -1$	δ_K $p = 4, 3, 2, 1, 0$	δ_K $p = -1$	δ_ε $p = 4, 3, 2, 1, 0, -1$
1:1	$-2.0- \quad - 3.4$	-13.4	$0.0-1.0$	$2.0- \quad 3.7$	17.9	$0.0- \quad -1.0$
1:2	$-2.0- \quad - 3.1$	$- 8.5$	$0.0-0.6$	$2.0- \quad 3.4$	10.2	$0.0- \quad -0.7$
1:3	$-2.0- \quad - 2.9$	$- 6.7$	$0.0-0.5$	$2.0- \quad 3.1$	7.9	$0.0- \quad -0.5$
1:4	$-2.0- \quad - 2.7$	$- 5.8$	$0.0-0.4$	$2.0- \quad 3.0$	6.6	$0.0- \quad -0.5$
2:2	$-3.9- \quad - 6.3$	-12.4	$0.0-0.6$	$4.2- \quad 7.0$	15.3	$0.0- \quad -0.6$
2:1	$-3.9- \quad - 8.1$	-19.4	$0.0-0.8$	$4.2- \quad 9.2$	27.8	$0.0- \quad -0.8$
3:1	$-6.1- \quad -13.6$	-25.4	$0.0-0.4$	$6.6-16.6$	39.0	$0.0- \quad -0.4$
4:1	$-8.6- \quad -19.3$	-32.3	$0.0-0.1$	$9.6-27.0$	54.2	$0.0- \quad -0.1$

Table 3. Deviation in per cent from correct value of K and ε, when an error of $\pm 2.0\%$ is induced into the initial concentrations of the component kept constant. $W = 1.0 \times 10^p$.

	$b' = b \times 1.02$			$b' = b \times 0.98$		
$m:n$	δ_K $p = 4, 3, 2, 1, 0$	δ_K $p = -1$	δ_ε $p = 4, 3, 2, 1, 0, -1$	δ_K $p = 4, 3, 2, 1, 0$	δ_K $p = -1$	δ_ε $p = 4, 3, 2, 1, 0, -1$
1:1	$0.0- \quad 1.6$	15.2	$-2.0- \quad -3.0$	$0.0- \quad - 1.5$	-11.9	$2.0-3.1$
1:2	$-2.0- \quad - 0.7$	5.7	$-2.0- \quad -2.6$	$2.0- \quad \quad 0.9$	$- 4.8$	$2.0-2.7$
1:3	$-3.9- \quad - 2.9$	1.5	$-2.0- \quad -2.5$	$4.2- \quad \quad 3.1$	$- 1.0$	$2.0-2.6$
1:4	$-5.8- \quad - 4.9$	1.7	$-2.0- \quad -2.4$	$6.3- \quad \quad 5.4$	2.0	$2.0-2.5$
2:2	$-1.9- \quad \quad 0.7$	8.3	$-2.0- \quad -2.6$	$2.0- \quad - 0.6$	$- 7.1$	$2.0-2.6$
2:1	$0.0- \quad \quad 4.8$	22.3	$-2.0- \quad -2.8$	$0.0- \quad - 4.4$	-16.4	$2.0-2.8$
3:1	$0.3- \quad \quad 9.5$	30.0	$-1.9- \quad -2.3$	$-0.3- \quad - 8.4$	-21.2	$2.0-2.4$
4:1	$1.1- \quad 14.9$	41.1	$-1.8- \quad -2.1$	$-1.1- \quad -12.9$	-27.2	$1.9-2.2$

DISCUSSION

From Table 1 and the tables not printed here, it can be found that the present method gives correct value for K and ε, when calculated for the correct compositions. Further, it is seen, that σ_K and ε are different for the different

calculations. If ε is known (and in some cases when ε is unknown), the composition can be found from one set of measurements when comparing with the tables. In the other cases, the best way to determine $m : n$ is to make two series of measurements, with A or B kept constant, respectively. For the correct composition, σ_K should have its minimum value, and ε should have the same value in the two series.

For the most stable complexes, *i.e.* $W = 0.1$, and especially for the compositions 3 : 1 and 4 : 1, it can be very difficult to obtain small values for σ_K for the correct composition, and little can be said from the calculation of this quantity. In this case, a good agreement in the calculated values for ε in the two series must be demanded if any conclusion about the composition can be drawn.

Tables 2 − 3 can give valuable information if the initial concentrations of the reactants are not exactly known, which may be caused by impure chemicals or variable water content. If the calculated values of K in the two series differ more than they should, according to the standard deviations, the results can be compared with Tables 2 − 3.

When using the new method it should be remembered that the concentrations must be chosen according to the optimum conditions.[2] This is important, because σ_K in most cases will have another value for other initial concentration ratios.

APPLICATIONS

The method was applied on three different complexes with known composition and conditional constants. The extinctions were measured with a Zeiss PMQ II spectrophotometer. When using Table 1 and the tables not printed here, it should be remembered that in these tables, it is always the initial concentration of the component corresponding to n (the composition is $m : n$) which is held constant.

Table 4. Calculations for the iron(III)-tiron system at pH = 1.12. The extinctions were measured at 660 nm in 1.0 cm cells.

	Trial composition $m : n$	Obtained conditional constant K	Standard deviation $\sigma_K(\%)$	Extinction coefficient $\varepsilon \times 10^{-3}$
Tiron = const.	1 : 1	4.19×10^2	2.2	1.56
	1 : 2		5.3	4.48
	1 : 3		6.3	8.81
	2 : 2		9.3	2.81
	2 : 1		26.3	1.25
Fe³⁺ = const.	1 : 1	3.93×10^2	2.3	1.47
	1 : 2		16.7	1.16
	2 : 2		8.2	2.65
	2 : 1		4.4	4.25
	3 : 1		5.2	8.36

(a) *The iron(III)-tiron system at* $pH = 1.12$. At $pH = 1.12$, tiron (1,2-dihydroxy-benzene-3,5-disulfonic acid, disodium salt) forms a 1 : 1 complex with iron (III).[4] Two series of measurements were made. The concentration of the component kept constant was 4.0×10^{-4} M, and the concentration of the other component was varied from 2.5 to 25.0 times in excess. The ionic strength was 0.1 M (KCl), and the extinctions were measured at 660 nm in 1.0 cm cells. The results of the calculations on these data are presented in Table 4. From this table, the conclusion must be a 1 : 1 complex. When considering the experimental error, the conditional constant is $K = 4.1 \times 10^2$. The latest reported value,[5] determined at $pH = 1.15$, is $K = 4.5 \times 10^2$. This seems to be a good agreement, since the constant varies a great deal with the pH-value.[4]

Table 5. Calculations for the iron(III)-tiron system at $pH = 10.10$. The extinctions were measured at 480 nm in 5.0 cm cells.

	Trial composition $m : n$	Obtained conditional constant K	Standard deviation $\sigma_K(\%)$	Extinction coefficient $\varepsilon \times 10^{-3}$
Tiron = const.	1 : 2		a	a
	1 : 3	1.12×10^{15}	3.7	6.84
	1 : 4		12.5	10.68
Fe^{3+} = const.	1 : 2		8.4	8.36
	1 : 3	1.05×10^{15}	7.4	6.66
	1 : 4		85.6	6.34

a No convergence.

(b) *The iron(III)-tiron system at* $pH = 10.10$. At $pH = 10.10$, iron(III) and tiron form a 1 : 3 complex.[4] To keep the iron dissolved, EDTA (ethylenediamine tetraacetic acid, di-sodium salt) was added in excess. Two different series were made. In the first one, the concentration of tiron was kept constant equal to 2.0×10^{-5} M, and the concentration of iron(III) was varied from 3.0×10^{-6} M to 8.0×10^{-5} M. In the second series, the concentration of iron(III)

Table 6. Calculations for the iron(III)-chrome azurol S system at $pH = 3.40$. The extinctions were measured at 570 nm in 5.0 cm cells.

	Trial composition $m : n$	Obtained conditional constant K	Standard deviation $\sigma_K(\%)$	Extinction coefficient $\varepsilon \times 10^{-3}$
	1 : 1		12.5	3.01
	1 : 2		55.5	1.68
Fe^{3+} = const.	2 : 2	1.21×10^{16}	5.0	4.15
	4 : 1		11.8	45.6

was kept constant equal to 2.0×10^{-6} M, and the concentration of tiron was varied from 3.0×10^{-4} M to 8.6×10^{-5} M. All solutions had a concentration of EDTA equal to 2.0×10^{-3} M, and the ionic strength was 0.1 M (KCl). The extinctions were measured at 480 nm in 5.0 cm cells. The results of the calculations on these data are presented in Table 5. From the two series, it must be concluded that the composition is 1:3, the conditional constant being $K = 1.1 \times 10^{15}$ when considering the experimental error.

(c) *The iron(III)-chrome azurol S system at pH = 3.40.* At pH = 3.40, iron(III) forms a 2:2 complex with chrome azurol S [6,7] (3″-sulfo-2″,6″-dichloro-3,3′-dimethyl-4′-hydroxyfuchson-5,5′-dicarboxylic acid). For this system, only one series of measurements was made. The concentration of iron(III) was kept constant equal to 6.0×10^{-6} M, and the concentration of chrome azurol S was varied from 0.5 to 3.5 times the concentration of iron(III). The ionic strength was 0.1 M (KCl), and the extinctions were measured against reagent blanks at 570 nm in 5.0 cm cells. The results of the calculations are presented in Table 6. Here, the conclusion must be a 2:2 complex with conditional constant $K = 1.2 \times 10^{16}$. For comparison, the value for the same constant published by Klausen [5] is $K = 2.8 \times 10^{16}$. For constants of this order of magnitude, the agreement seems to be satisfactory.

REFERENCES

1. Lang, R. P. *J. Am. Chem. Soc.* **84** (1962) 1185.
2. Norheim, G. *Acta Chem. Scand.* **23** (1969) 2808.
3. Topping, J. *Errors of Observation and their Treatment*, 3rd Ed., Chapman and Hall, London 1962.
4. Schwarzenbach, G. and Willi, A. *Helv. Chim. Acta* **34** (1951) 528.
5. Klausen, K. S. *Anal. Chim. Acta* **44** (1969) 377.
6. Klausen, K. S. and Langmyhr, F. J. *Anal. Chim. Acta* **28** (1963) 335.
7. Langmyhr, F. J. and Klausen, K. S. *Anal. Chim. Acta* **29** (1963) 149.