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Spectrophotometric evaluation of stability constants of 1:1 weak complexes from continuous variation data

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Abstract

A bilogarithmic hyperbolic cosine method for the spectrophotometric evaluation of stability constants of 1:1 weak complexes from continuous variation data has been devised and applied to literature data. A weighting scheme, however, is necessary in order to take into account the transformation for linearization. The method may be considered a useful alternative to methods in which one variable is involved on both sides of the basic equation (i.e. Heller and Schwarzenbach, Likussar and Adsul and Ramanathan). Classical least squares lead in those instances to biased and approximate stability constants and limiting absorbance values. The advantages of the proposed method are: the method gives a clear indication of the existence of only one complex in solution, it is flexible enough to allow for weighting of measurements and the computation procedure yield the best value of log β_{11} and its limit of error. The agreement between the values obtained by applying the weighted hyperbolic cosine method and the non-linear regression (NLR) method is good, being in both cases the mean quadratic error at a minimum.

Keywords: Stability constants; Weak complexes; Continuous variation; Spectrophotometry

1. Introduction

For systems with two active components S and L the net change in a variable frequently is plotted against a second variable to obtain (de Maine, 1965) maxima or minima. The equilibrium $S + L \rightleftharpoons SL$, may serve as a useful model (Connors, 1987; Polster and Lachmann, 1989; Huang, 1982; Blanda et al., 1989; de Fontaine et al., 1977) for investigating, e.g. the interaction between a metal and a ligand, an anion and an enzyme, the binding of a ligand with a protein to form a complex or the entrapment of an organic anion between the hydrophobic cavity of a macrocyclic host (Boccio et al., 2006; Rippe, 1997). The continuous variation method (McBryde, 1974; Sommer and Langova, 1988) is based on plotting the measured absorbance, or another physical property related with concentration (Homer and Perry, 1986; Gill and Olivera, 1990), against the mole fraction of one component of the complex for mixtures of the two reactants, S and L, the sum of the concentrations being kept constant. When the molar solutions of S and L are equimolecular the diagrams, which have a pseudotriangular shape, are generally used to determine the stoichiometry of the complex; no additional information being extracted from equimolar diagrams. Nevertheless, for single 1:1 weak complexes, equimolar diagrams can be readily analyzed (Bruneau et al., 1992; Adsul and Ramanathan, 1978) to give the stability constant without recourse to non-equimolar diagrams.

The method of continuous variation data from Ostromisslensky (1911), Denison (1912) and Job (1928), and an enormous literature concerning this topic has been generated (Beck and Nagypal, 1990; Facchiano and Ragone, 2003; Havel et al., 1977; Huang, 1982; Klausen and Langmuir, 1963; Slovak and Borak, 1974; Vives et al., 2000). The application of classical least squares based methods with variables not well separated (Adsul and Ramanathan, 1978; Likussar and Boltz, 1971; Likussar, 1973; Meloun et al., 1988) leads to biased results in the values of stability constants. For this reason, the non-linear relationship between the absorbance and the mole ratio is transformed into a linear one in which the explanatory (independent) variable x may be taken as an exact quantity, free of any error. A bilogarithmic hyperbolic cosine method for the evaluation of stability constants of 1:1 weak complexes has been devised, and applied to spectrophotometric continuous variation data reported in the analytical literature.

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The present paper forms part of an investigation into the uses (Asuero, 1989, 1992, 1993) of hyperbolic relationships in parameter estimation.

2. Basic equations

Let us assume that a solution containing a known equilibrium concentration of substance S is allow to react with L being partly converted to SL, $S + L \rightleftharpoons SL$. The stability constant for reaction in term of absorptiometric quantities is given (constant ionic strength, *I*, temperature and medium is assumed) by

$$\beta_{11} = \frac{[\mathrm{SL}]}{[\mathrm{S}][\mathrm{L}]} = \frac{A/\varepsilon}{(T_\mathrm{S} - A/\varepsilon)(T_\mathrm{L} - A/\varepsilon)} \tag{1}$$

where *A* represents (McBryde, 1974) the corrected measured or "excess absorbance" (1 cm cells) at a given wavelength (λ) (equivalent to the absorbance of the sample solution minus the absorbance of a hypothetical solution of S and L which is identical in every way except that it contains no complex), and T_S and T_L the total concentrations of the S and L, respectively, and ε is the molar absorptivity of the SL complex. Eq. (1) may be rearranged to give

$$\frac{X(1-X)}{A} = \frac{1}{T_0^2 \varepsilon \beta_{11}} + \frac{1}{T_0 \varepsilon} - \frac{A}{\varepsilon^2 T_0^2} \tag{2}$$

where $T_0 = T_\mathrm{S} + T_\mathrm{L}$, and

$$X = \frac{T_\mathrm{S}}{T_0} = \frac{T_\mathrm{S}}{T_\mathrm{S} + T_\mathrm{L}} \tag{3}$$

(T_0 being maintained constant throughout measurements). If measurements are performed with light path *l* cm, *A*/*l* should be substituted for all values of *A* in the following discussion. If the curves are symmetrical with X_max around 0.50 and $A_{\lambda_1}/A_{\lambda_2}$ values of the various solutions in each case are found to be constant, it is concluded that 1:1 complexes are forming exclusively (Connors, 1987; Polster and Lachmann, 1989) under these conditions.

3. Weighted bilogarithmic hyperbolic cosine method

By multiplying Eq. (2) through by $\varepsilon T_0/\sqrt{X(1-X)}$ we get

$$\frac{\varepsilon T_0 \sqrt{X(1-X)}}{A} + \frac{A}{\varepsilon T_0 \sqrt{X(1-X)}} = \frac{(1/T_0\beta_{11}) + 1}{\sqrt{X(1-X)}} \tag{4}$$

and taking into account the definition of hyperbolic cosine, $\cosh x = (e^x + e^{-x})/2$, then

$$2\cosh\left(\ln\left(\frac{A}{\varepsilon T_0 \sqrt{X(1-X)}}\right)\right) = \frac{(1/T_0\beta_{11}) + 1}{\sqrt{X(1-X)}} \tag{5}$$

By simple algebra it may be easily shown from Eq. (5) that

$$\log\left(\cosh\left(\ln \frac{A}{2A_\mathrm{lim}\sqrt{X(1-X)}}\right)\right) + \log 2 = \log\left(\frac{1}{T_0\beta_{11}} + 1\right) - \log\sqrt{X(1-X)} \tag{6}$$

where A_lim is the absorbance corresponding to complete formation at $X = 0.5$ (Havel et al., 1977),

$$A_\mathrm{lim} = A_{\mathrm{lim},0.5} = \frac{\varepsilon T_0}{2} \tag{7}$$

Thus, a representation of the left hand of Eq. (6) against $\log\sqrt{X(1-X)}$ should give a straight line $Y = a_0 + a_1 x$ of -1 slope. The value of the unknown parameter β_{11} can be calculated from the intercept a_0 of this line, which is obtained by the weighted least squares method (Asuero et al., 2006; Sayago and Asuero, 2004) as

$$\beta_{11} = \frac{1}{T_0(10^{a_0} - 1)} \tag{8}$$

The merits of Eq. (6) is that the variable *A* is found only in the left side, and that the right hand may be considered as exactly known, being on this way prone to the correct application of weighted least squares.

4. Weights

The non-linear relationship between absorbance *A* and the mole ratio *X* is intrinsically linear because it is possible to make a transformation of both variables such that the relationship between the transformed variables can be expressed as a straight line. The dominant random experimental errors are typically concentrated in the measured initial absorbances and concentration may be considered free from error. Then, as *A* data are converted into transformed data *Y*, the weighting factors w_i for the weighted least squares based on the use of Eq. (6) are given by (Asuero and González, 1989; Gort and Hoogerbrugge, 1995)

$$w_i = \left(\frac{1}{\partial Y/\partial A}\right)^2 = (\ln 10)^2 A^2 \coth^2\left(\ln \frac{A}{2A_\mathrm{lim}\sqrt{X(1-X)}}\right) \tag{9}$$

where $\coth x = \cosh x/\sinh x = (e^x + e^{-x})/(e^x - e^{-x})$

The weights calculated as indicated in Eq. (9) may be normalized by transforming each "old" weight into a new one, w^*, in such a way that, in being *k* the number of points on the graph we have

$$\sum w = k \tag{10}$$

and

$$w^* = \frac{kw}{\sum w} \tag{11}$$

From Eq. (9) we may derive

$$w_i/C = y^2 \coth^2\left(\ln\left(\frac{y}{2\sqrt{X(1-X)}}\right)\right) \tag{12}$$

where $y = A/A_\mathrm{lim}$ and $C = (\ln 10)^2 A_\mathrm{lim}^2$. In Fig. 1 (left and right), the logarithm of w_i/C and w_i/C as a function of *X*, are depicted, for varying values of $\beta_{11}T_0$ (inset). The greater the values of the $\beta_{11}T_0$ product and *X*, the greater is the corresponding weight.

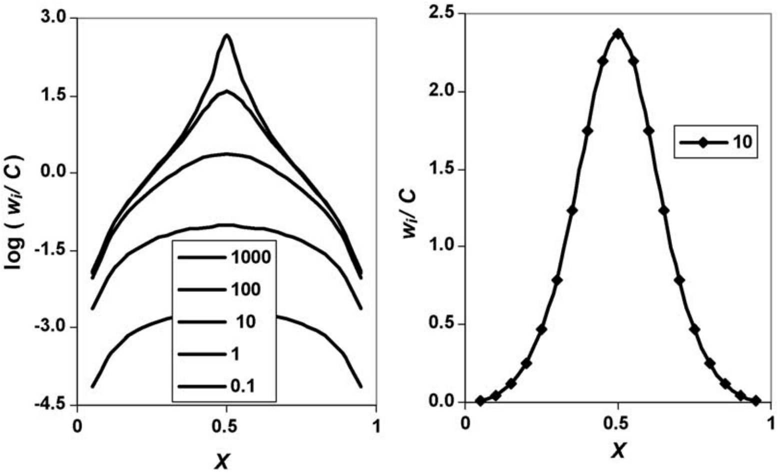

Fig. 1. (Left) Graphic representation of the logarithm of the weighting factors by C (where $C = \ln^2 10 A_{\mathrm{lim}}^2$) against X, the mole ratio of S for mixtures of S and L, $X = T_S/(T_S + T_L)$, for varying $\beta_{11}T_0$ values: 1000, 100, 10, 1 and 0.1, for the top to the bottom curves, respectively. (Right) Graphic representation of the weighting factor by C against X in for the $\beta_{11}T_0$ equal to 10 case.

5. Choice of the limit absorbance

Some authors have recommended to obtain the maximum absorbance at $X = 0.5$, A_{lim}, by measuring the absorbance of a solution containing a definite excess of ligand L and a concentration of S of $T_0/2$ against a reference solution containing an identical ligand concentration (Likussar and Boltz, 1971). This method, however, is not applicable if the excess of reactant absorbs strongly or if the SL complex is too weak. In fact, a degree of complexation of 99% at $X = 0.5$ requires the ratios of concentration of L to S given in Table 1.

As A_{lim} is not accessible to direct measurement a procedure for overcoming this difficulty must be followed before Eq. (6) can be applied. From Eqs. (2) and (7) we get the expression (Sayago et al., 2005)

$$\frac{X(1-X)}{A} = \frac{1}{2A_{\mathrm{lim}}}\left(\frac{1}{T_0\beta_{11}} + 1\right) - \frac{A}{4A_{\mathrm{lim}}^2} \tag{13}$$

This is essentially the same as the relation obtained for 1:1 complexes by Heller and Schwarzenbach (1951). Representation of $X(1-X)/A$ against A should gave a straight line of the kind $Y = a_0 + a_1 x$ (least squares analysis) from which

$$A_{\mathrm{lim}} = \frac{1}{2}\sqrt{\frac{-1}{a_1}} \tag{14}$$

Table 1
Ratio of T_L/T_S required for a 99% complex formation at $X = 0.5$

$\beta_{11}T_0$	T_L/T_S
0.1	991
0.5	199
1	100
5	20.8
10	10.9
100	2

$$\beta_{11} = \frac{1}{T_0(a_0\sqrt{-1/a_1} - 1)} \tag{15}$$

Attention must be given to Eq. (13) because the A variable is involved on both sides. Then, an error in this quantity appears in both coordinates mutually correlated in both conditions, that is, the independent variable x is not an exact quantity and the independence of errors is not fulfilled (Exner, 1997; Sayago et al., 2004) For these reasons by applying Eq. (13) we obtain and approximate and biased value of the A_{lim} which must be further refined as indicated in the following.

The procedure suggested in order to improve the A_{lim} value chosen involves the following steps: (i) calculate an estimate value of A_{lim} from Eqs. (13) and (14); (ii) vary A_{lim} systematically around the first estimate value obtained in (i) and apply the entire procedure in each case; (iii) take the best value of β_{11} as that which minimize the mean quadratic error in absorbance measurements

$$\sigma = \sqrt{\frac{\sum (A - A_{\mathrm{est}})^2}{N}} \tag{16}$$

where N is the number of A–X data pairs and A_{est} is calculated from Eq. (17)

$$A = A_{\mathrm{lim}}\left(\frac{1}{T_0\beta_{11}} + 1 - \sqrt{\left(\frac{1}{T_0\beta_{11}} + 1\right)^2 - 4X(1-X)}\right) \tag{17}$$

once the values of A_{lim} and β_{11} are known. A BASIC program run on a TOSHIBA model Satellite M40-285 personal computer was written to perform this task. The program carries out automatically all necessary calculations. A printout of the program is available from the authors. Alternatively, the absorbance corresponding to the crossing point of the extended tangents ($X \approx 0$ and $X \approx 1$) or limiting slopes (Slovak and Borak, 1974) or even the largest absorbance value, A' at $X = 0.5$ may be taken as starting values for the minimization process.

6. Applications

The weighted bilogarithmic hyperbolic cosine method has been applied to several chemical systems (Table 2). Experimental details and absorbance versus X data employed are given in that follows:

I. Iron(III)-chromotropic acid (Heller and Schwarzenbach, 1951). $T_0 = 8 \times 10^{-4}$ M; $\lambda = 655$ nm. Data pairs $\{X,A\}$: 0.1, 0.117, 0.2, 0.219, 0.3, 0.304, 0.4, 0.360, 0.5, 0.381, 0.6, 0.362, 0.7, 0.307, 0.8, 0.224, 0.9, 0.118.

II. Iron(III)-8-hydroxyquinoleine (Sandell and Spindler, 1949; Brewer, 1980). $T_0 = 1.19 \times 10^{-3}$ M, $\lambda = 645$ nm; pH = 1.92. Data pairs $\{X,A\}$: 0.5, 0.169, 0.6, 0.163, 0.7, 0.141, 0.8, 0.106, 0.9, 0.058.

III. Zinc(II)-methylglyoxal bis(4-phenyl-3-thiosemicarbazone) (Herrador, 1985). $T_0 = 6 \times 10^{-5}$, $\lambda = 460$ nm. Data pairs $\{X,A\}$: 0.913, 0.090, 0.833, 0.167, 0.750, 0.247, 0.667, 0.317, 0.583, 0.361.

IV. Silver(I)-dithizone (Likussar and Boltz, 1971; Likussar, 1973). $T_0 = 5 \times 10^{-5}$ M, $\lambda = 485$ nm. Data pairs $\{X,A\}$: 0.1, 0.110, 0.2, 0.216, 0.3, 0.317, 0.4, 0.415, 0.5, 0.470, 0.6, 0.410, 0.7, 0.322, 0.8, 0.215, 0.9, 0.110.

V. 3-Aminopyridine-picric acid (Bruneau et al., 1992; Harris, 1995). $T_0 = 10^{-4}$ M. Data pairs $\{X,A\}$: 0.1, 0.106, 0.2, 0.214, 0.3, 0.311, 0.4, 0.402, 0.5, 0.442, 0.6, 0.404, 0.7, 0.318, 0.8, 0.222, 0.9, 0.110.

VI. Iron(III)-thyocianate (Brewer, 1980). $T_0 = 10^{-3}$ M, $\lambda = 500$ nm. Data pairs $\{X,A\}$: 0.417, 0.460, 0.5, 0.473, 0.583, 0.460, 0.667, 0.415, 0.750, 0.339, 0.833, 0.237, 0.917, 0.125.

The calculated values for the various systems subject of study are given in Table 2. Errors were obtained in each case (Asuero et al., 1988) by applying random error propagation law. In the case of the weighted hyperbolic cosine procedure, i.e. from Eq. (8) we get

$$s^2_{\log\beta_{11}} = \frac{\partial(\log\beta_{11})}{\partial\beta_{11}} s^2_{\beta_{11}} = \frac{1}{\ln^2 10\beta^2_{11}} \left(\frac{\partial\beta_{11}}{\partial a_0}\right)^2 s^2_{a_0} \qquad (18)$$

and then as

$$s_{\beta_{11}} = \frac{\ln 10 s_{a_0} 10^{a_0}}{T_0(10^{a_0} - 1)} \qquad (19)$$

we have finally

$$s_{\log\beta_{11}} = \frac{10^{a_0} s_{a_0}}{10^{a_0} - 1} \qquad (20)$$

The non-linear option NL-REGR 881212 of Ebert et al. (1989) (multidimensional Newton method with partial derivatives calculated numerically) run on a personal CASIO FX-880 computer was applied with Eq. (18) as user defined function, as a reference method, for the sake of comparison. In the case of complexes of low and moderate stability, the experimental curve can be quite flat in the vicinity of the maximum. Figs. 2 and 3 show the application of the weighted bilogaritmic method to the iron(III)-chromotropic acid system. For complexes with $\log(\beta_{11}T_0) > 1.5$, the intercepts a_0 of the corresponding regression lines are included into brackets in Table 2 instead of the standard deviation of the $\log\beta_{11}$ values. The results obtained by applying the method of Heller and Schwarzenbach are biased and approximate, because the variables are not well separated, as A figures on both sides of Eq. (9). The Heller and Schwarzenbach approach tends, in general, to overestimate the $\log\beta_{11}$ values and underestimate the A_{lim}, whereas the bilogarithmic method shows no apparent systematic deviation (Fig. 4). The agreement between the values obtained by the weighted hyperbolic cosine

Table 2
Evaluation of stability constants of 1:1 weak complexes

System	Parameter	Eqs. (14) and (15)	This paper	NLR[a]
(I) Fe(III)-chromotropic acid	$\log\beta_{11}$	3.678 ± 0.029	3.673 ± 0.022	3.674 ± 0.022
	A_{lim}	0.775 ± 0.015	0.777	0.777 ± 0.012
	σ	1.478E−03	1.476E−03	1.476E−03
(II) Fe(III)-8 hydroxy-quinoleine	$\log\beta_{11}$	2.961 ± 0.040	2.925 ± 0.011	2.913 ± 0.047
	A_{lim}	0.605 ± 0.049	0.633	0.643 ± 0.040
	σ	4.993E−04	4.579E−04	6.148E−04
(III) Zn(II)-methylglyoxal bis(4-phenyl-3-thiosemicarbazone)	$\log\beta_{11}$	5.358 ± 0.071	5.336 ± 0.136	5.318 ± 0.050
	A_{lim}	0.560 ± 0.017	0.564	0.567 ± 0.010
	σ	2.208E−03	2.121E−03	2.101E−03
(IV) Silver(I)-dithizone	$\log\beta_{11}$	5.986 ± 0.081	6.159 [0.0052]	6.203 ± 0.044
	A_{lim}	0.561 ± 0.018	0.551	0.548 ± 0.003
	σ	4.383E−03	2.492E−03	2.407E−03
(V) 3-Aminopyridine-picrid acid	$\log\beta_{11}$	5.576 ± 0.177	5.497 [0.0074]	5.514 ± 0.046
	A_{lim}	0.561 ± 0.018	0.567	0.566 ± 0.005
	σ	3.343E−03	3.208E−03	3.154E−03
(VI) Iron(III)-thiocyanate	$\log\beta_{11}$	3.527 ± 0.033	3.497 ± 0.031	3.484 ± 0.036
	A_{lim}	1.013 ± 0.017	1.037	1.049 ± 0.031
	σ	2.552E−03	2.392E−03	2.384E−03

[a] Non-linear regression.

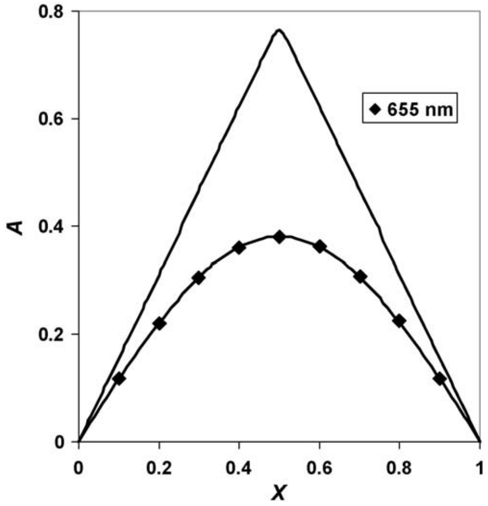

Fig. 2. Continuous variation data for the Fe(III)-chromotropic acid system: absorbance, A, at (◆) 655 nm vs. X, the mole ratio of Fe(III) for mixtures of Fe(III) and chromotropic acid. $T_0 = T_S + T_L = 8 \times 10^{-4}$ M. The curve in the figure is calculated with β_{11} and A_{lim} given in Table 2, the solid line corresponds to a complex with the same A_{lim} and degree of complexation (formation) unity: $T_0\beta_{11} > 10^4$.

method and the non-linear regression is good, being in both cases the mean quadratic error at a minimum (Fig. 5).

The method proposed is independent of the molecular structure of the species or of the chemical nature of the complexation and gives a clear indication of the existence of only a single complex in solution (a_1 slope equals to -1). It is flexible enough to allow the weighting of measurements and the computation procedure yield the best value of $\log \beta_{11}$, and its limit of error, and may be considered a useful alternative to methods in which one variable is involved on both sides of the basic equation, i.e. Heller and Schwarzenbach (1951), Likussar (1971) or Adsul and Ramanathan (1978).

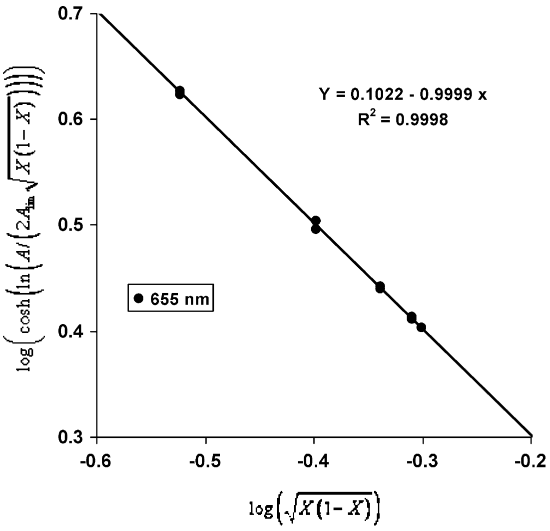

Fig. 3. Weighted bilogarithmic hyperbolic cosine method applied to the Fe(III)-chromotropic acid system at (●) 655 nm. Inset are the corresponding weighted least squares equation and the coefficient of determination, R^2.

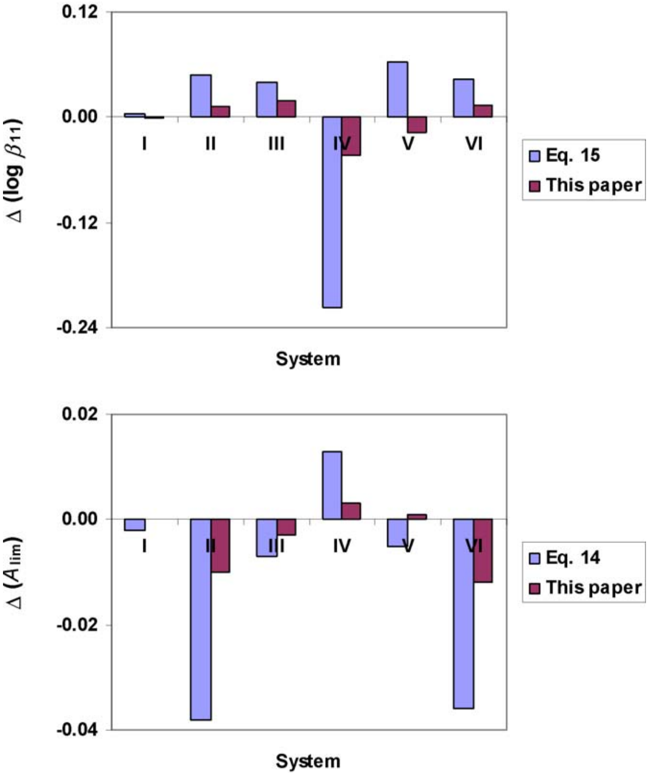

Fig. 4. (Top) Differences in the $\log(\beta_{11}T_0)$ values obtained by the Heller and Schwarzenbach method (Eq. (15)) and the weighted bilogaritmic method (this paper) with respect to the non-linear regression (NLR) method, taken as reference, $\Delta\log\beta_{11} = |\log\beta_{11}|_{\text{this paper}} - |\log\beta_{11}|_{\text{NLR}}$. (Bottom) Differences in the A_{lim} values obtained by the Heller and Schwarzenbach method (Eq. (15)) and the weighted bilogaritmic method (this paper) with respect to the non-linear regression (NLR) method, taken as reference, $\Delta A_{\text{lim}} = |A_{\text{lim}}|_{\text{this paper}} - |A_{\text{lim}}|_{\text{NLR}}$.

It should be recalled that these equations are applied only if 1:1 complexes are formed. Deviations of slope from its nominal value could indicate a problem with the model: for example, more than one binding site per molecule or more than one ligand bound per site (Li et al., 1988). For either a 1:1 or 2:2 interactions, the continuous variation plot is symmetrical and has a maximum at 0.5 when A is plotted against X. However, it is

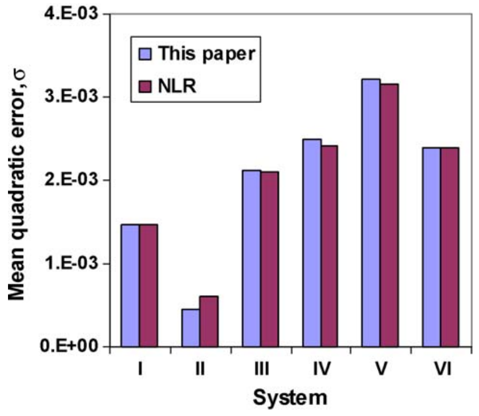

Fig. 5. Graphical comparison between the mean quadratic error of absorbance measurements, σ, Eq. (17) for the weighted bilogarithmic method (this paper) and then non-linear regression (NLR) method taken as reference.

possible to distinguish theoretically and experimentally 1:1 and 2:2 continuous variation curves (Sayago et al., 2005; Woldbye, 1955). The curve for the S_2L_2 complex, e.g. exhibits inversions with corresponding concave parts and parabolic approaches to the end-points (Klausen and Langmuir, 1963, 1968; Sayago et al., 2005). The measurements under various stoichiometric dilutions of solutions and for various path of the absorbing solution are the suitable playground for getting necessary data (Havel et al., 1977). However, if the method of continuous variation is applied to complex formation reaction that take place stepwise, plots can be obtained in which the existence of intermediate complexes is obscured (Huang et al., 2003; van der Linden and Beers, 1974), if no proper caution is observed.

A major goal of scientific experimentation is the discovery of relationships among variables (Draper and Smith, 1981). The evaluation of stability constant by linearized plots on this respect seems to be more prevalent, probably owing to the transparency of the methods used (Fuchs and Gessner, 2001), in spite of the modern possibility of using black-box computer programs. Non-linear least squares on the other hand, are not always problem-free. Occasionally, problems arise because of the choice of the data, initial estimates, convergence or multiple local minima, all typical of non-linear regression (Nievergelt, 1994). The weighted bilogarithmic hyperbolic tool devised here, for all reasons indicated above, constitutes an appropriate and useful mathematical model and a method of choice for the study of complexes by the continuous variation method.

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