

A Straightforward Method to Determine Equilibrium Constants from Spectrophotometric Data

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Spectrophotometry is an excellent experimental method providing sufficient information on the equilibrium concentrations of a (usually liquid) reaction mixture. For many decades, chemists have made a great effort to infer equilibrium constants from this rich source of information. A paradox feature of this effort was that they usually did not aim at *maximum or optimum information* concerning the equilibrium constant but tried to fulfil peculiar conditions to be able to use one of the numerous *simplified treatments* to evaluate experimental data. The common feature of all these simplifications was to remove the essentially non-linear nature of the related model equations, to select and manipulate experimental data in such a way that finally, to get the equilibrium constant, only linear models had to be treated.

Problems arising from this "selection and manipulation" of the experimental data have been discussed in detail by Richard Ramette (1) in this journal. He also proposed – among others – a clear and correct treatment of the linear models, described criteria to choose the most appropriate one to handle experimental data, and developed a computer program – as early as 1967 – to infer equilibrium constants from practically all experimental "cases". However, his treatment also remained in the framework of *linearized models* only. Almost two decades later, Michael Collins extended Ramette's algorithm to use with other methods in addition to spectrophotometry (*e. g.*, solution calorimetry, NMR chemical shift) (2), still keeping the essentially linearized evaluation of the experimental data. Though there have been several applications of non-linear parameter estimation in evaluation of spectrophotometric and other equilibrium measurements, they were based on the mathematical and numerical treatment of the particular nonlinear model (3,4). A critical overview of early chemical equilibrium applications of non-linear parameter estimation is given in Ref. (3). However, earlier applications usually had to deal with many numerical and computer programming details, being too much tedious to implement in undergraduate lab courses.

The present paper – after another decade – shows a non-linear evaluation method for spectrophotometric data. This method uses the physical description *as it is* for a model function to infer the equilibrium constant from experimental data. In 1986, Collins mentioned, "in favor of the Ramette approach" the "widespread microcomputer availability". Nowadays, thanks to the widespread availability of sophisticated mathematical software packages, non-linear parameter estimation became an easy task to make with minimum mathematical and no programming skill. The availability of the non-linear methods makes it possible to concentrate on optimum information when calculating the equilibrium constant, with the additional convenience of almost no mathematical manipulation of the underlying (simple)

equations. Details of the numerical techniques of non-linear parameter estimation can be found in current statistical or numerical textbooks (5-7).

Derivation of the model

As the aim of this paper is mainly to show the ease and superiority of non-linear methods, we shall only discuss simple cases and show a few examples. Let us consider the prototype donor-acceptor reaction, with the same notation as in Refs. 1 and 2:



We are interested in the equilibrium constant of this reaction, which can be written as

$$K = \frac{[DX]}{[D] \cdot [X]} \cdot \frac{f_{DX}}{f_D \cdot f_X}, \quad (2)$$

where brackets stand for equilibrium concentrations, and the symbols f indicate the corresponding activity coefficients. Keeping in mind that activity coefficients are usually calculated or determined independently of the spectrophotometric experiment, it is sufficient to determine a "simplified version" of K , the equilibrium quotient:

$$Q = \frac{[DX]}{[D] \cdot [X]} \quad (3)$$

This is the quantity we are interested to infer from spectrophotometric data, which consist of absorbances measured at different wavelengths, of mixtures with varied concentrations. The absorbance of an equilibrium mixture at a wavelength λ can be written in terms of Beer's Law:

$$A^\lambda = \ell \cdot (\varepsilon_D^\lambda \cdot [D] + \varepsilon_X^\lambda \cdot [X] + \varepsilon_{DX}^\lambda \cdot [DX]) \quad (4)$$

When applying the straightforward method to determine the equilibrium quotient, Eq. (4) is directly used as a model function; we apply a non-linear parameter estimation algorithm to estimate ε_D^λ , ε_X^λ , ε_{DX}^λ and Q , as parameters of the A^λ function.

Let us show what are the independent variables in the A^λ function, and how Q appears in it as a (non-linear) parameter. The preparation of equilibrium mixtures proceeds in one of two ways. We can either mix the two components of the complex D and X in known initial concentrations, or mix the complex DX and one of its constituents (say, X). In the first case, be c_D and c_X the initial concentrations mixed. At equilibrium, we get the complex concentration $[DX]$. From the stoichiometry of reaction (1) it follows that

$$[D] = c_D - [DX] \quad (5a)$$

$$[X] = c_X - [DX] \quad (5b)$$

The resulting absorbance (divided by the optical path-length ℓ) can be written as:

$$\frac{A^\lambda}{\ell} = \varepsilon_D^\lambda \cdot (c_D - [DX]) + \varepsilon_X^\lambda \cdot (c_X - [DX]) + \varepsilon_{DX}^\lambda \cdot [DX] \quad (6)$$

Rearranging terms, we get:

$$\frac{A^\lambda}{\ell} = \varepsilon_D^\lambda \cdot c_D + \varepsilon_X^\lambda \cdot c_X + (\varepsilon_{DX}^\lambda - \varepsilon_D^\lambda - \varepsilon_X^\lambda) \cdot [DX] \quad (7)$$

Now, the unknown equilibrium concentration $[DX]$ can be obtained solving the equation analogous to Eq. (3):

$$Q = \frac{[DX]}{(c_D - [DX]) \cdot (c_X - [DX])} \quad (8)$$

The solution provides

$$[DX] = \frac{1 + Q \cdot (c_D + c_X) \pm \sqrt{1 + 2Q \cdot (c_D + c_X) + Q^2 \cdot (c_D - c_X)^2}}{2Q} \quad (9)$$

The second case, with c_{DX} and c_X initial concentrations mixed, gives only somewhat different formulae as a result. The equilibrium concentrations in this case can be written as

$$[DX] = c_{DX} - [D] \quad (10a)$$

$$[X] = c_X + [D] \quad (10b)$$

The only formal difference with respect to the previous case comes from the sum in Eq. (10b) instead of the two differences of Eq. (5a and 5b). This results in the equation

$$Q = \frac{c_{DX} - [D]}{[D] \cdot (c_X + [D])} \quad (11)$$

to solve. The solution is

$$[D] = \frac{1 + Q \cdot c_X \pm \sqrt{1 + 2Q \cdot (c_X - 2c_{DX}) + Q^2 \cdot c_X^2}}{2Q} \quad (12)$$

This should be substituted in the corresponding Beer's expression:

$$\frac{A^\lambda}{\ell} = \varepsilon_D^\lambda \cdot [D] + \varepsilon_X^\lambda \cdot (c_X + [D]) + \varepsilon_{DX}^\lambda \cdot (c_{DX} - [D]) \quad (13)$$

Rearranging, and substituting expression (12) gives:

$$\frac{A^\lambda}{\ell} = \varepsilon_X^\lambda \cdot c_X + \varepsilon_{DX}^\lambda \cdot c_{DX} + \left(\varepsilon_X^\lambda + \varepsilon_D^\lambda - \varepsilon_{DX}^\lambda \right) \frac{1 + Q \cdot c_X \pm \sqrt{1 + 2Q(c_X - 2c_{DX}) + Q^2 c_X^2}}{2Q} \quad (14)$$

Putting together Eqs. (7) and (9), we get, for the other case:

$$\frac{A^\lambda}{\ell} = \varepsilon_X^\lambda c_X + \varepsilon_D^\lambda c_D - \left(\varepsilon_X^\lambda + \varepsilon_D^\lambda - \varepsilon_{DX}^\lambda \right) \frac{1 + Q \cdot (c_D + c_X) \pm \sqrt{1 + 2Q(c_D + c_X) + Q^2 \cdot (c_D - c_X)^2}}{2Q} \quad (15)$$

From Eqs. (14) and (15) it is easily seen that, in both cases, A^λ/ℓ is the dependent variable, as a function of either c_X and c_D , or c_X and c_{DX} (or c_D and c_{DX} , which leads to the same result) as the independent variables. As *parameters* of the function appear ε_D^λ , ε_X^λ , ε_{DX}^λ and Q . However, while A^λ/ℓ is a linear function of ε^λ -s, it is a non-linear function of Q — hence the necessity for a non-linear parameter estimation.

To determine the appropriate wavelength, we have to keep in mind that — as can be seen from Eqs. (14) and (15) — the term containing Q is multiplied by $\varepsilon_X^\lambda + \varepsilon_D^\lambda - \varepsilon_{DX}^\lambda$, so that the information content of the absorbance concerning the equilibrium quotient is the greater, the bigger the difference between molar absorptivities of the constituents and the complex. (This is evident; if $\varepsilon_X^\lambda + \varepsilon_D^\lambda = \varepsilon_{DX}^\lambda$, there is no change in absorbance due to the complex formation.) Parameter estimation is efficient if the number of measured data points largely exceed the number of parameters to estimate. In the above case, there are four parameters to determine, so a desirable number of data points is about 20 or more. If it is problematic to prepare so many mixtures of different concentrations, we have to measure at several wavelengths. At each wavelength, three molar absorbances appear as additional parameters, but the equilibrium quotient should be the same. So if we have only 10 mixtures, measuring their absorbances at four different wavelengths results in 13 parameters but 40 measured experimental points. The number of degrees of freedom can be calculated (see *e.g.* Ref. 5) as (the number of measured points) – (the number of parameters) – 1. This gives 15 for 20 mixtures at a single wavelength and 26 for 10 mixtures at 4 wavelengths. The latter case usually gives better precision in the parameter estimation, as the number of degrees of freedom is higher.

However, there is another possibility to increase the precision of estimation. If we suppose some absorption band profile (*e.g.* Gaussian or Lorentzian) for the three species involved, then we have three parameters (the position of the absorption maximum, the maximum absorption or the area of the band, and the width of the band) for each absorbing species, plus the equilibrium quotient. These are ten parameters altogether, but the number of parameters does not increase if we measure absorbances at several wavelengths. With the above example, measuring 10 mixtures at four different wavelengths means 40 data points and ten parameters, *i. e.*, 29 degrees of freedom, compared to 26 when we ignored the absorption

band shape. Of course, there is no limit for increasing the number of test wavelengths. When doing so, we not only determine the equilibrium quotient to a high precision, but also get the complete absorption spectra of all the three species involved (if they do have nonzero absorptivities in the observed wavelength region).

There is another advantage of using the above-described straightforward non-linear method with the absorption band parameters and a large number of data points at many test wavelengths. If we suspect additional equilibria with additional species instead of the simple complex formation described by Eq. (1), we can easily rewrite our model including the absorption parameters of additional species, with the appropriate solution of the corresponding equilibrium concentration(s), and check for the improvement of the fit due to the more complicated model. When there is a significant improvement, we can opt for the more complex model and interpret its parameters to describe the equilibrium.

Statistical method

In this section, we would like to overview, in a few words, the way to get estimates of the parameters and their errors in a non-linear estimation process. Without going into mathematical details, we would rather like to explain how the machinery works.

Non-linear parameter estimation can be considered as a synonym for curve fitting to a data set, if the data are not arranged along a line. The most widely used method to fit an appropriate curve to such data is called *the method of least squares*. According to this, if we have data points $A_{\text{obs},i}$ and a function to model the data which gives the fitted values at each data point $A_{\text{calc},i}$, we get optimal fit of the curve – and hence optimal values of the parameters involved in the model functions – if we minimize the sum

$$S = \sum_{i=1}^m w_i (A_{\text{obs},i} - A_{\text{calc},i})^2 \quad (16)$$

called the *weighted residual sum of squares*. The summation goes over all the m measured points. The factor w_i is the so-called weighting factor. If we choose w_i the way that it is proportional to $1/s^2(A_{\text{obs},i})$, the inverse of the variance at each measured point, we get the best kind of estimates called *minimum variance unbiased*. Most commercial software packages do not have the option to enter individual weighting factors, so they use equal weights at each data point (*i.e.* $w_i = 1$ at all i), which is equivalent to the approximation that the experimental error is the same at each measurement. (This is, in fact, a *non-weighted* estimation. For the sake of simplicity, we shall also use this approximation in the examples treated below.) Now, if the experimental error is really constant at each point, whenever we transform the measured data in a nonlinear way (*e.g.* make a nonlinear transformation of the original data to get a linearized function), we should also transform the weight function, which then becomes different at each data point. The transformation of errors can be done with the method usually

called *the propagation of errors*, which has been treated at length in this journal (8, 9, 10). The weighting problem is easily avoided using a nonlinear parameter estimation method with the original observed values. The reader is advised to refs. 5-7. to learn more about weighting and the method of non-linear least squares.

Most estimation methods readily provide also an estimate of the error of the parameters. However, this error indicates a measure of reliability of the parameters, which is dependent on the number of experimental points that have been used in the estimation procedure. A better measure of the reliability is the *confidence interval* of the parameters, which takes into account both the calculated error and the number of data points. If the *standard deviation* of a parameter p is $s(p)$ – which is simply given by most software packages as “error” –, the corresponding interval at $1 - \alpha$ level of confidence is

$$p \pm s(p) t_{\nu}(1 - \alpha/2) , \quad (17)$$

where $t_{\nu}(1 - \alpha/2)$ is that value of the variable t_{ν} of a Student’s t -distribution, at which the cumulative distribution function is exactly $1 - \alpha/2$. The subscript ν is the number of degrees of freedom (see previous section).

Application

To show the details of how this method works, we have chosen two examples. First we re-analyze the data of Table 3 in the paper of Ramette, and compare our results with those originally reported by the author. The second example is an undergraduate experiment in the physical chemistry practical course at our university, the charge-transfer complex formation between I_2 and DMSO in a CCl_4 solution. For this latter case, we will analyze an undergraduate lab experiment with measurements at two selected wavelengths first, and also a wide-range wavelength study of this equilibrium with the determination of the relevant absorption bands, to show the validity of the equilibrium model.

As the first illustration of the non-linear parameter estimation method for spectrophotometric data, we re-evaluate Ramette's calculation of equilibrium quotient for the simple complex-forming reaction between Fe^{3+} (substance D), and SCN^{-} (substance X), keeping the original assumption that a 1:1 complex ion is the only product in a perchloric acid medium for this reaction at the wavelength measured (450 nm), and the only absorbing species is $FeSCN^{2+}$, (substance DX). This case is modelled by Eq. (15), where ϵ_D^{λ} and ϵ_X^{λ} (the molar absorption coefficients of Fe^{3+} and SCN^{-}) are put to zero, ϵ_{DX}^{λ} and Q are the parameters to estimate. Note that the model function is simply the measured absorbance as a function of composition, there is no need to manipulate raw experimental data. The graph of the fitted model function (15) is shown in Fig. 1, where the excellent quality of the fit is also obvious.

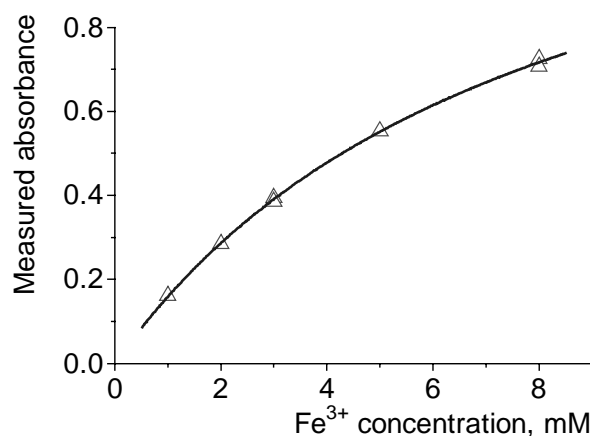


Figure 1. Plot of the fit of Eq. (15) to the experimental data points (triangles) for the Fe^{3+} - SCN^- complex formation measured at 450 nm. (The initial SCN^- concentration is 0.0003 M.) Note that the plot shows the raw data without any transformation.

We performed the same analysis adding first the observations at 440 nm, then also those at 460 nm, taken from the original report of the experimental data by Frank and Oswalt (11). The nonlinear method also provides reasonable estimates of the errors and confidence intervals. As we can see from the results shown in Table 1, increasing the number of experimental data points from 7 to 21 results in decreasing the half width of the confidence interval of the equilibrium quotient from 15 to 6, compared to $Q = 131.0$, *i. e.*, from a relative error of $\approx 12\%$ to $\approx 4.6\%$. (When performing the non-linear fit at each single wavelength separately, the respective half-widths were 13, 15 and 11. This means that the decrease is really due to the increase of the degrees of freedom with increasing number of data points.)

Table 1. Precision of the estimated parameters as a function of the number of experimental data points. Experimental data are taken from Ref. (11). Absorbances of the same seven mixtures are measured at each wavelength.

Nº of data points	Nº of degrees of freedom	95 % confidence interval of Q
7 (450 nm)	4	116-146
14 (440+450 nm)	10	122-139
21 (440+450+460 nm)	16	125-137

The estimated *correlation* of the equilibrium quotient to the estimated molar absorptivities of the complex is -0.95 at all the three wavelengths, which explains the relatively high uncertainty of the estimated value of Q . The comparison with Ramette's original results can also be explained on the basis of this correlation. In table 3 of ref. 1, Ramette gives $EDX =$

3552.5 (to compare to $\epsilon_{DX} = 3609.9$ obtained in the present study), and an “average Q ” of 134.6, (to compare to 131.0). This is in accordance with the high negative correlation of the two parameters; the lower EDX parameter gives rise to a higher Q value. Ramette does not calculate the error of EDX , and does not take it into account in the calculation of the error of Q , so his “average deviation” calculated for Q , 1.6 % or 2.15 largely underestimates the actual standard deviation (6.2) obtained in this study. This latter gives rise to the 95 % confidence interval 116-146, given in the first row of Table 1. Nevertheless, the reported $Q = 134.6$ is within these confidence limits.

The second example comprises a thorough analysis of experimental data concerning the formation of a 1:1 charge transfer complex of iodine and DMSO in CCl_4 . We prepared nine mixtures containing 0.001 M iodine each, and varying amounts of DMSO in the concentration range 0.01 M - 0.09 M. First we analyzed absorbance data of the nine mixtures at two wavelengths (446 nm and 518 nm; at about the maxima of the complex and the iodine absorption bands), measured with a Perkin-Elmer $\lambda 15$ spectrophotometer. This case is also modelled by Eq. (15), with ϵ_D^λ (the molar absorption coefficient of DMSO) put to zero, so that there are altogether 5 parameters to estimate, including Q .

Table 2. Estimated parameters of the charge-transfer formation equilibrium $\text{I}_2 + \text{DMSO}$. Nine mixtures were measured at two wavelengths, to give 18 experimental points.

parameter	mean value	standard deviation
Q	9.7	1.1
$\epsilon_{\text{I}_2}^{446} / \text{M}^{-1} \text{cm}^{-1}$	133.1	12
$\epsilon_{\text{complex}}^{446} / \text{M}^{-1} \text{cm}^{-1}$	1484	72
$\epsilon_{\text{I}_2}^{518} / \text{M}^{-1} \text{cm}^{-1}$	950	9
$\epsilon_{\text{complex}}^{518} / \text{M}^{-1} \text{cm}^{-1}$	142	45

Results of this analysis in Table 2 show that the precision of the parameters is quite satisfying. Molar absorptivities of the iodine obtained this way agree very well with those determined from pure I_2 dissolved in CCl_4 . (Performing the non-linear fit at the two wavelengths separately, the 95 % confidence intervals of Q obtained are 6.3-12.2 at 446 nm and 8.2-19.7 at 518 nm, compared to 7.3-12.2 obtained with the simultaneous fit to both datasets. This illustrates that estimated equilibrium quotients from single datasets might be close to one or the other end of the confidence interval of the simultaneous fit.)

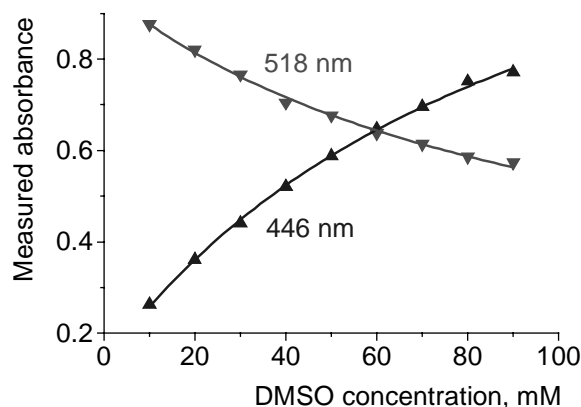


Figure 2. Plot of the fit of Eq. (15) to the experimental data points (triangles) for the I_2 - DMSO charge-transfer complex formation. 446 nm is the maximum of the complex absorption band, 518 nm is that of the I_2 band. The initial I_2 concentration is 0.001 M throughout.

We also analyzed a large set of data measured for the nine mixtures at 106 wavelengths from 400 nm to 530 nm, supposing Gaussian band shapes for both absorbing species. To get the corresponding 954 data points does not take that much time; making a digital data collection at equidistant wavelengths is a routine procedure with modern spectrophotometers. The Gaussian band shape can be written in the following, general form:

$$\varepsilon_G(\lambda) = \frac{A}{w\sqrt{\pi/2}} \exp \left[-\frac{2 \left(\frac{K}{\lambda} - \frac{K}{\lambda_{\max}} \right)^2}{w^2} \right] \quad (18)$$

where λ is the wavelength, λ_{\max} is the wavelength at the maximum of the band, and the constant $K = 1239.84 \text{ eV}\cdot\text{nm}$ is to convert wavelength to energy in eV units. (The band shape should be calculated as a function of energy, instead of wavelength.) Accordingly, w , the width of the band at its half height is also in eV units. A is the area of the band in corresponding units. This function is added to the model equation (15) to replace the appropriate ε_λ values.

Parameters thus obtained are listed in Table 3, indicating a remarkable precision of the equilibrium constant and the spectral parameters as well. Figure 3a shows the fit of Eq. (15) combined with the Gaussian band to the measured absorbances.

Table 3. Estimated parameters of the charge-transfer formation equilibrium I₂ + DMSO. Nine mixtures were measured at 106 wavelengths, to give 954 experimental points. Absorption spectra of the I₂ and the charge transfer species were calculated as Gaussian bands. Band parameters are from Eq. (18).

parameter	mean value	standard deviation
Q	12.9	0.14
$\lambda_{\max}(\text{I}_2) / \text{nm}$	516.6	0.13
$A(\text{I}_2)$	438.4	1.23
$w(\text{I}_2) / \text{eV}$	0.367	0.001
$\lambda_{\max}(\text{complex}) / \text{nm}$	447.2	0.10
$A(\text{complex})$	693.1	2.1
$w(\text{complex}) / \text{eV}$	0.409	0.001

It is readily seen that the 1:1 complex equilibrium together with the two Gaussian band shapes describes the studied experimental data fairly well, but there seem to be some *systematic errors*, *i. e.*, the fitted curves seem to deviate systematically from the experimental data especially in the range of 400 to 450 nm. The calculated contribution of the two species and the their sum is shown in Figure 4a for one of the best-fit series. Here, the deviation is also evident as a slightly s-shaped systematic difference in the same wavelength range.

One possible explanation for this systematic difference is that the complex band cannot be fitted with a Gaussian, so we also tried another model, fitting the linear combination of a Gaussian and a Lorentzian function to the complex band, but leaving the I₂ band as a Gaussian, since there wasn't any s-shaped deviation in that part of the spectrum. The Lorentzian function can be written in a similar form, using the same variables and parameters as with the Gaussian function:

$$\varepsilon_L(\lambda) = \frac{2A}{\pi} \frac{w}{4 \left(\frac{K}{\lambda} - \frac{K}{\lambda_{\max}} \right)^2 + w^2} \quad (19)$$

In this second model function, we added x_L percent of the Lorentzian and $1 - x_L$ percent of the Gaussian to get the absorbance of the complex band, so that the absorptivity is

$$\varepsilon_{\text{com}}^\lambda = x_L \cdot \varepsilon_L(\lambda) + (1 - x_L) \cdot \varepsilon_G(\lambda) \quad (20)$$

Results thus obtained are shown in Table 4., and the fit of the composite band function can be seen in Figure 3b.

Table 4. Estimated parameters of the charge-transfer formation equilibrium $I_2 + \text{DMSO}$ for the same data as in Table 3. Here, the absorption spectrum of the complex was calculated as a linear combination of a Lorentzian and a Gaussian band. Band parameters are from Eqs. (18), (19), and (20).

parameter	mean value	standard deviation
Q	11.8	0.10
$\lambda_{\max} (I_2) / \text{nm}$	516.0	0.11
$w(I_2) / \text{eV}$	0.364	0.0009
$A(I_2)$	434.3	1.01
$\lambda_{\max} (\text{Gauss, complex}) / \text{nm}$	449.3	0.38
$w(\text{Gauss, complex}) / \text{eV}$	0.371	0.004
$\lambda_{\max} (\text{Lorentz, complex}) / \text{nm}$	408.0	1.3
$w(\text{Lorentz, complex}) / \text{eV}$	0.376	0.044
$A(\text{complex})$	745.8	11.8
$x_L (\text{Lorentz percentage})$	15.6	3.3

Here, there is no evidence of systematic error, so this excellent fit can be accepted as an adequate model for the description of the charge transfer complex formation equilibrium. Comparing the 95 % confidence interval obtained with this obviously reliable overall fit (11.6-12.1) to that obtained from the two datasets of Fig. 2 (7.3-12.2), we can see that there is an important improvement of the precision, and the reliable overall fit supports the higher Q values from the global fit. We would like to emphasize at this point that the fit including band shapes is essentially non-linear, so it would not have been possible to get such a global fit using linearized methods.

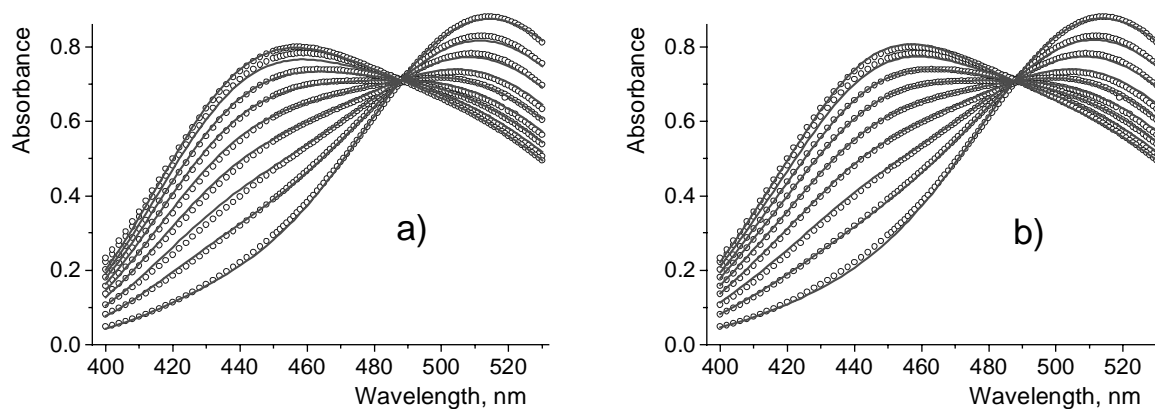


Figure 3. a) Plot of the fit of Eq. (15), with molar absorptivities calculated from Gaussian band parameters according to Eq. (18), to the measured absorbances of a series of nine mixtures of 0.01 to 0.09 M DMSO and 0.001 M I_2 in CCl_4 solution. Open circles are measured experimental points; solid curves show the fitted function. Note the s-shaped systematic deviation of the fitted curves in the lower wavelength range. b) Same plot with fitted curves using linear combination of Gaussian and Lorentzian bands according to Eq. (20). There are no s-shaped systematic deviations in this plot.

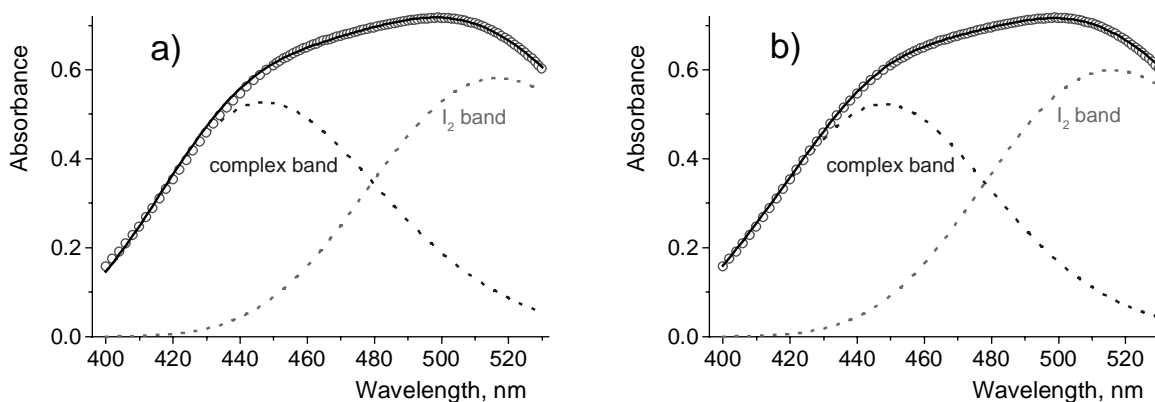


Figure 4. Plots of the fit for the single mixture of 0.04 M DMSO from Figure 3, with the contribution of the two absorbing species. The presence in a) and the absence in b) of the s-shaped systematic deviation is clearly seen.

As a hint for actual laboratory application of the method, we would propose to evaluate the results of any 1:1 complex equilibrium lab experiment, using raw spectrophotometry data, with Eq. (14) or (15), optionally adding – in case of data measured in a wide wavelength range – the calculation of molar absorptivities according to Eqs. (18), (19) or (20).

Worked examples

As we have pointed out earlier, the aim of this paper is also to show the ease of use of non-linear parameter estimation models with modern software applications. We have selected Origin 5.0 from Microcal Software to prepare worked examples with. This selection is due to the excellent graphical options and the versatile non-linear fitting capabilities of this product, together with the free availability of a demo version, which includes full features of the product and complete manuals as well. Studying the Origin project files, the reader can have an interactive fitting session, which we hope could develop some skill in non-linear fitting.

Available project files include the material of Figure 1 (simple fit), Figure 2 (multiple fit) and Figure 3 and 4 (complicated band model fits and the calculation of the contributing bands). To see the examples, you need to download Microcal Origin Version 5.0 Demo from the <http://www.microcal.com> web site (unless you already have Version 5.0). (The present version is 6.0, available at the <http://www.OriginLab.com> web site. All the examples work also with this version.) Once you will have installed the software, you need to create a subdirectory in the parent directory containing the file Origin50.exe (or Origin60.exe, respectively), called "NonLinQ". Then you need to download the contents of this NonLinQ subdirectory from the Journal site. The ReadMe.txt file contains the listing of all the files you need to have in this subdirectory. Having done all this, you have to open the Example.OPJ project file from Origin, and *activate at least the button "Step 1"*. This will automatically customize Origin so that all example projects are ready to run. The first example (Example.OPJ) contains both a layout window and a notes window, where you can find explanations to study the project. All further examples contain only notes windows with explanations.

Here is a short description of what can be studied with the project files included.

Example.OPJ customizes Origin to run this and the other examples (Step1 button), and describes in details how to perform a non-linear curve fit, with data from Figure 1. The procedure can be done either using the appropriate commands in Origin, or simply pushing four buttons one after another and watching what happens.

2wFit.OPJ illustrates how to perform a multiple fit (fit a function simultaneously to multiple set of data) with data from Figure 2.

GbFit.OPJ and *GLbFit.OPJ* show the fit of complicated functions containing the shape of two absorption bands to a large dataset with data from Figure 3.

Gbands.OPJ and *GLbands.OPJ* show the calculation of the bands of two absorbing species contributing to the measured spectrum. Results of these calculations are shown in Figure 4.

Conclusion

We would like to recall some properties of the non-linear method. It is simple and straightforward in the sense that there is no need to manipulate measured absorbances (except a practical normalization to 1 cm path length), we can measure at concentrations and wavelengths where we get maximum information for the equilibrium constant – regardless of the applicability of linear approximations –, and we can use the same model function in the entire concentration range.

A further great advantage is that we can include analytical – or even numerical – expressions for the individual components' absorption band shapes and get a global description of the equilibrium in a wide wavelength range and at arbitrarily large number of different wavelengths. This latter helps not only to increase the precision of the equilibrium constant, but provides the components spectra as well. In addition to improve the quality of interpretation of analytical and optical inference, these features have obvious pedagogical advantages. There are plenty of possibilities – especially in practical courses – to implement the non-linear evaluation method, thus offering a unique interpretation and explanation of the concept of chemical equilibria and spectral contributions.

ACKNOWLEDGEMENT

The authors acknowledge assistance in the preparation of the example projects of Peter Holpár and András Somogyi, as well as the kind technical advice of the Microcal staff. E. Keszei is a beneficent of the grant OTKA T019396 from the Hungarian Research Fund.

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