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Investigation of equilibria in solution. Determination of equilibrium constants with the HYPERQUAD suite of programs

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Abstract

A new suite of 10 programs concerned with equilibrium constants and solution equilibria is described. The suite includes data preparation programs, pretreatment programs, equilibrium constant refinement and post-run analysis. Data preparation is facilitated by a customized data editor. The pretreatment programs include manual trial and error data fitting, speciation diagrams, end-point determination, absorbance error determination, spectral baseline corrections, factor analysis and determination of molar absorbance spectra. Equilibrium constants can be determined from potentiometric data and/or spectrophotometric data. A new data structure is also described in which information on the model and on experimental measurements are kept in separate files.

Keywords: Equilibrium constant; Potentiometry; Solutions; Spectrophotometry

1. Introduction

The determination of equilibrium constants is an important process for many branches of chemistry [1]. Developments in the field of computation of equilibrium constants from experimental data were reviewed a few years ago [2,3]. Since that time, many more programs have been published, mainly so as to be able to use microcomputers for the computations. In this paper, we concentrate on the elaboration of potentiometric and spectrophotometric data; the most commonly used programs for solution equilibrium constant determination are given in Table 1.

All of these programs use a least-squares approach, the principles of which have been expounded in detail [4]. Many programs follow what we may call a standard approach. In this, the sum of squares is minimized by means of the Gauss–Newton–Marquardt algorithm. The derivatives required by this algorithm were originally obtained numerically, but following Nagypal et al. [5] some programs used derivatives calculated analytically. The free concentrations are calculated by solving the non-linear simultaneous equa-

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tions of mass balance using the Newton–Raphson method. Potentiometric data points are weighted by formula that allows for greater pH errors in the region of an end-point than elsewhere. For absorbance data points a relative weighting scheme is used.

Some noteworthy exceptions are as follows. Minimization using pit-mapping (LETAGROP [6,7] and ABLET [8] families), Davidon–Fletcher–Powell method (STEW [9]), direct search (EY608 [10]), the method of steepest descent (CFTSP [11]) orthogonal decomposition (CLINP [12], ELORMA [13], SIRKO [14]). MINUITS [15] used regression equations of the Padé type. In DHMINOPT [16] there are three minimization routines and if one using derivatives fails the Nelder–Mead simplex method is called automatically. Solution of the mass-balance equation using a bisection algorithm (CFTSP [11]) or "another procedure which iterates by systematically modified increments" (MAXIPOT-F [17]) or a projection method due to Bugaevsky (SIRKO [14]). There have been attempts to make corrections for activity variation (SCOGS2a [18], SPECA [19]) and to use extended Debye–Hückel theory (DCLET [8] and BSTAC [20], an augmented version of SUPERQUAD [21]). Refinement of stoichiometric indices is included as an option in POLET [22] and SQUAD84 [23]. ECORM [24] was a computer implementation of the old (pre-computer) graphical methods, as are KATCOM [25] and Sidrak and Aboul-Seoud's program [26].

PKAS [27] is notably original in many respects. As it treats only protonation/deprotonation reactions (cf TITRA [28], TITAN [29]), it is possible to apply procedures that cannot be applied in the more general case. Thus, there are only two mass-balance equations in the two unknowns [L] and [H], say. [L] is eliminated to leave a polynomial in [H] which is solved by Newton–Raphson iteration. The minimization involves some kind of direct search method which, unfortunately, was not specified in detail. The program is highly interactive and requires much input of chemical knowledge. Having said that, the authors claim that the program never crashes. BEST [30] also employs a direct search method described as follows: "perturb a small fraction of the basis set [of refinable parameters] by an increment, see the effect on the sum of squares [of residuals in pH] and repeat in an appropriate direction to affect further improvement". This sounds like something akin to a simplex minimization.

Perhaps the most interesting developments have been the two attempts to write programs that can handle *any* equilibrium data. SIRKO [14] has a universal response function and a system whereby analytical derivatives are generated for the Jacobian. MICMAC [31] requires the programming of a module (MOSP) specific to a particular type of experimental data, but because of this numerical derivatives must be employed in the Jacobian.

Our own work has had two fundamental motivations: to provide a robust program for the computation of equilibrium constants and to tackle the problem of model selection. Although MINIQUAD [32,33] was robust, and is still being used, Motekaitis and Martell [30] have articulated the criticism, of which we were only too well aware, that the function minimized did not involve experimentally observed quantities directly. This was put right in SUPERQUAD [21], which therefore had the significant advantage of providing a statistically sounder basis for model selection.

The problem of model selection arises with systems in which it is impossible, a priori, to state what chemical species will be present in appreciable concentrations in the reaction mixtures. In those cases there is a need for some criteria both to determine whether or not the computed equilibrium model is satisfactory in a chemical sense and to see if the calculated data points agree with those observed, within experimental error. We have discussed these criteria previously [21]. What emerges from the analysis is that the expectation value for the sample variance will be unity if, and only if, a weight matrix is used that is the inverse of the variance–covariance matrix of the experimental data [4].

Now, in order to help with the model selection process, it seems a good idea to make use of more than one kind of measurement simultaneously on the same set of reaction solutions. We therefore have developed HYPERQUAD, which can determine equilibrium constants from both potentio-

Table 1
Computer programs used for calculating equilibrium constants from potentiometric (V) and spectrophotometric (A) data

Program	Ref.	Data type[a]	Program	Ref.	Data type[a]
ABLET	[8]	A	MINIQUAD75	[33]	V
ACBA	[55]	V	MINISPEF	[67]	A
ACREF3AM	[56]	V	MINUITS	[15]	V
AP	[57]	V	MUCOMP	[68]	V
Asuero et al.	[58]	A	MUPROT	[69]	V
BEST	[30]	V	Nievergelt	[70]	A
BSTAC	[20]	V	NONLIN15	[71]	V, A
CFTSP	[11]	A	Papanastasiou and Zogas	[72]	V
CLINP	[12]	A	PHODEC	[73]	A
DALSFEK	[59]	V, A	PKAS	[27]	V
DCLET	[8]	A	POLET	[22]	V
DCMINOPT	[60]	A	PROTAF	[74]	V
DHMINOPT	[16]	V	PSEQUAD	[75]	V, A
ECORM	[24]	V	SCOGS2a	[18]	V
ELORMA	[13]	A	SCOGS2b	[76]	V
EQNMR	[61]	N	Sidrak and Aboul-Seoud	[26]	V
ESAB2M	[62]	V	SIRKO	[14]	G (C)
EY608	[10]	A	SPECA	[19]	A
HYPERQUAD	[34]	V, A	SPECFIT	[77]	A (E)
HYPNMR	[36]	N	SPFAC	[48]	A (E)
KATCOM	[25]	V	SQUAD	[78]	A
LETAGROP–SPEFO	[6]	A	SQUAD84	[23]	A
LETAGROP–VRID	[7]	V	STAR	[52]	A
LITRA	[63]	A	STBLTY	[79]	V
LOGMIN	[64]	V	STEW	[9]	V
MAXIPOT-F	[17]	V	SUPERQUAD	[21]	V
MICMAC	[31]	G (V, P, N)	TITAN	[29]	V
MINIGLASS	[65]	V	TITFIT	[80]	V
MINIMIX	[66]	A	TITRA	[28]	V
MINIQUAD	[32]	V	Wentworth et al.	[81]	A

[a] Additional data types used in calculations: E, ESR; C, Calorimetry; G, general; N, NMR; P, Polarography.

metric and spectrophotometric (absorbance) data. However, working simultaneously on different kinds of data makes it imperative that a full, rigorous weighting scheme is used. Only when this is so will it be possible to apply simple statistical tests for the goodness of fit, with an expectation value of unity for the sample variance. In order to implement this procedure we have developed a new means of estimating the error in a measure of absorbance.

We have previously outlined the main features used in the HYPERQUAD program [34]. Since that publication, we have developed a suite of inter-related programs which are directed towards data preparation, preliminary model exploration, simulation and preparation of speciation diagrams. Together these programs provide almost all the facilities which have previously been used on an ad hoc basis in the process of mode selection. The one exception is that we have not explicitly included an electrode calibration program. We assume that electrodes will be calibrated by means of a strong acid–strong base titration, which HYPERQUAD can handle. MAGEC [35] is a program designed for electrode calibration.

Another set of programs in the HYPERQUAD suite, HypNMR, is described elsewhere [36]. The principal program in that set can be used to determine equilibrium constants from NMR chemical shift data. It is assumed that the species in equilibrium are in rapid exchange on the NMR time-scale so that each value of chemical shift is

the concentration-weighted average of the shifts of the individual species.

2. The HYPERQUAD suite of programs

2.1. The data structure

It became clear very early on that the treatment of different kinds of experimental data logically required a well defined data structure. Previously all programs had used a single file for all the data. With a suite of programs, however, the data files serve as input to different procedures and must therefore be standardised. Conceptually there are four kinds of data file as follows.

(i) A model file. This file contains only the data relevant to a chemical model, viz. the names of the reagents, the equilibrium constants defined by initial value and stoichiometric coefficients, keys to indicate refinable quantities and whether or not a species absorbs light. In addition, our model file may hold the wavelengths at which measurements were made and any known molar absorbances. The model files are given the extension PAR.

(ii) Potentiometric data file. We have Instrument Potentiometric Data (extension IPD) and Program Potentiometric Data (extension PPD) files. The former are ASCII data files such as may be produced by an automatic titrator whilst the latter are suitable for HYPERQUAD. There is a facility in the data editor (HEDIT) for converting IPD to PPD. The PPD file contains the conditions (total amounts, burette concentrations, etc.) of a titration curve as well as the titration data (volume added, e.m.f. of pX measurement).

(iii) Spectrophotometric data. Instrument Absorbance Data (extension IAD) files are ASCII data files typically produced by a spectrophotometer, that is, they contain an absorbance spectrum. Three forms are at present acceptable: initial wavelength and increment, followed by absorbance values, wavelength and absorbance value pairs or the standard JCAMP-DX format [37,38]. A Base Absorbance Data (.BAD) file contains the concentrations, etc., used when the spectrum was recorded and it holds a complete spectrum in HYPERQUAD format. A Program Absorbance Data file (extention PAD), on the other hand will contain absorbance data at wavelengths selected in the HydraSP program, and may hold data in the form of a spectrophotometric titration curve of batch data.

(iv) Output files. These are files produced during the computations. For example, HYPERQUAD produces a standard output file OUTPUT.RES which may be used in post-run processing of the results of a computation by means of the program HANDOUT.

The fact that the model files do not hold experimental data is a great advantage. For example, it means that one model file may be used with different data sets without the duplication that would have arisen with, say, SUPERQUAD [21]. Furthermore, the model file serves as input to the speciation program HYPHEN where experimental data are irrelevant. Other ways in which the data structure is advantageous are in the determination of end-points (program HENNA) where the model is irrelevant and in factor analysis (program HydraSP), where again the model is irrelevant.

2.2. Linking potentiometric and spectrophotometric data

Each titration curve or experimental spectrum is assigned a unique label. Absorbance Data files carry a second label which may or may not be the same as the label of a potentiometric data file. If this second "corresponding to" label is the same as one in the potentiometric data file then the concentrations, etc., are taken from that file. In this way, it is possible to process either potentiometric or spectrophotometric data on its own, or to process them together.

HYPERQUAD is distributed with a large number of specimen data files. Also, there is a set of template files which can be used for entering new data.

2.3. The HYPERQUAD data editor

The HYPERQUAD data editor HEDIT is designed to make data entry as chemist-friendly as possible. In fact, when this facility is used as

standard, users will not need to know how the data are actually stored in the data files. The data present in a model file (PAR), a potentiometric and/or spectrophotometric data file (PPD, BAD or PAD) are offered for editing in an intelligible form, i.e. reagents are identified by the names specified in the model file, quantities and concentrations are clearly labelled as such, and so forth.

There are two limitations at present to the type of data that may be handled in HEDIT. No more than two electrodes are allowed. Few people have made measurements using two electrodes, and no-one (at least to our knowledge) has yet made measurements using more. The maximum number of reagents is determined by screen resolution and is 12 at present. Otherwise, if the amount of data is more than can appear at one time on the screen there are screen-paging options. One great advantage of using HEDIT is that the user does not require any instructions on how to imput data to HYPERQUAD, although specifications are included in the documentation should anyone prefer to use a text editor.

In addition to being able to process Program data files, the data editor can convert Instrument data files to Program form. Also, if a BAD file corresponds to a single species in solution, this can be input into the model file in the form of a set of molar absorbances. For example, the spectrum of a metal ion could be recorded and transferred into the model file as just described. This is possible because the BAD file holds the concentration of the species which give rise to the spectrum, thus permitting the calculation of molar absorbance from the measurements.

2.4. The programs JOIN and SUPPER

JOIN is a utility program that links the data from more than one titration curve into one data file. We have kept this as a separate program, rather than include the option in HEDIT, for this reason. The experimental data for any one titration will be stored in a file which is the primary record of the experimental data. Files containing data from more than one titration curve are secondary sources of data. SUPPER will take SUPERQUAD [21] data and convert it into PPD form.

2.5. The program Haber

This program is concerned with the estimation and treatment of errors in absorbance measurements. It is well known that errors in absorbance measurements may depend on the absorbance value. For example, when using a photomultiplier the signal error is often assumed to be proportional to the signal value. However, it is also clear that the magnitude of errors will depend on instrument settings. Therefore, we have devised a scheme whereby the error can be expressed as a simple empirical function that can be incorporated in a model file.

First it is necessary to collect a number of spectra obtained under identical conditions. This means that not only must the instrument conditions be the same but also the spectrum must not vary with time. We prefer to use solid samples such as doped glass filters for this purpose. Suppose n spectra have been obtained; we suggest $n = 9$ as a minimum. Then the abosrbance value is given by the mean of the n measurements and an estimate of the error at that absorbance value is given by the standard deviation of the n measurements:

$$\bar{A} = \frac{\sum_{j=1,n} A_j}{n}$$

$$s_A = \sqrt{\frac{\sum_{j=1,n} (A_j - \bar{A})^2}{n-1}}$$

The values of s_A are plotted on the screen as a function of $\bar{A}$ and the whole set of data is approximated by a simple four-parameter function. The first parameter specifies the function type, either quadratic or exponential. For a quadratic function we have $E = a + bA + cA^2$ and for the exponential function $E = a + be^{cA}$. The values of the parameters a, b and c may be adjusted interactively. It is possible to set one or more of these parameters to zero if that is required.

It may be noted that there is no attempt made to allow for the correlation that undoubtedly exists between the errors at different wavelengths in the same spectrum. Some evidence for this

correlation will be seen in the plots of s_A against $\bar{A}$ in the form of two values of s_A for some values of $\bar{A}$: there is one value when the absorbance curve has a positive slope and another when it has a negative slope. In any case one would normally choose an error function that passes through the middle of the data. We have taken the view that since the weights are only approximate, it is not worth correcting for correlation of errors.

The four parameters of the chosen error function are then entered into a model file. It is possible to have a different error function at each wavelength (thus allowing for the use of different detectors at different wavelengths) or that the error function is the same at all wavelengths.

In a HYPERQUAD calculation, the error at each absorbance value is calculated automatically from the error function in the model file and this is used to construct an approximate variance–covariance matrix for the absorbance measurements, from which the weights are derived. (see below)

2.6. The program HydrapH

This program takes as input a model file and a program Potentiometric Data file and is to be used for preliminary examintion of potentiometric data. The program offers 10 facilities. Six of these relate to presentation of the experimental data on the screen, i.e. e.m.f. or pH on the y-axis, volume, pH or a user-defined coordinate on the x-axis. Then there are species distrubution plots both as formation percentages relative to the total concentration of one reagent and as logarithms of concentration.

At least two programs have been already published concerning the simulation of titration curves, ESTA [39] and SCALPO [40]. We have included the interactive simulation of the titration curve(s) in HydrapH. In this, data in the model file may be amended in any way desired and either the calculated/observed titration curves are displayed or the difference (observed pH − calculated pH). It does not matter if the experimental readings are in mV or pH units. In parallel with the changes in the simulated titration curve there will also be changes in the speciation diagrams. Thus, it is possible to make a thorough preliminary analysis and ascertain both that the model is reasonable and that all the species postulated to be present and are in appreciable concentrations.

2.7. The program Henna

This is another program for the preliminary analysis of potentiometric data, concerned with end-point determination. The traditional methods used for end-point determination have limitations. Gran's method [41] fails for overlapping end-points [42]. First-derivative methods fail if the data are relatively sparse. For these reasons, we have developed a new algorithm in which the titration curve is fitted, by the method of least squares, by B-spline functions. A derivative of the B-spline function is used to determine the position of the end-point (maximum slope in the first derivative, zero in the second derivative, etc.). A similar procedure has been used to fit spectra [43]. For the purposes of the present application, the user needs to make only two choices: first the derivative required, the degree of the spline function being automatically chosen so that the derivative is a quadratic spline function, and second the number of B-spline pieces. These choices are made interactively. Optimal knot positions are determined automatically by repeated use of procedure called NEWNOT [44] and the observed/calculated titration curves are displayed along with the residuals and the desired derivative. After the user has indicated the approximate position of the end-point to be determined, its optimum position is calculated.

The only problem with this method arises when the number of data points in an interval between knots becomes equal to the degree of the spline function. In that case the calculated titration curve passes exactly through the observed data points, usually with wild oscillations. Since this does not normally happen near an end-point, it is not a serious problem for end-point determination.

2.8. The program HydraSP

This is the main program to be used for preliminary analysis of spectrophotometric data. It takes as input a model file and up to 28 spectra in the

form of BAD files. It offers five main facilities, as follows.

(i) Scaling and correction of data. Spectra can be scaled to equal concentration and path length in order to reveal isosbestic points. Scaling to equal areas can be used for the same purpose and will reveal isosbestic point in some circumstances where the simple scaling does not do so [45]. There is an offset facility which can be used to remove some baseline errors from the spectra. It works as follows. Assuming that the absorbance at the nth wavelength, λ_n, can be written as

$$A_{\lambda_n} = l \sum_j \varepsilon_{\lambda_n j} c_j + B$$

where B is a constant baseline error, it follows that if the absorbance at one wavelength, λ_b, is subtracted from data at all wavelengths the Beer–Lambert law will still be obeyed but a constant baseline error will have been eliminated:

$$A_{\lambda_n} - A_{\lambda_b} = l \sum_j (\varepsilon_{\lambda_n j} - \varepsilon_{\lambda_b j}) c_j$$

The penalty for using this facility is that the molar absorbances are composite but if λ_b is chosen in a region where there is little absorbance this will be of little significance. Therefore, we recommend that spectra be recorded if at all possible with data near the true baseline. To make our procedure more robust we use a smoothed value for the absorbance to be subtracted.

(ii) Selection of wavelengths for HYPERQUAD calculation. This is an interactive procedure so that the user can choose those wavelengths most suitable for the calculation. Now, in principle, data at one wavelength are sufficient for the purpose of calculating equilibrium constants. One would seek that wavelength which exhibits the maximum change in absorbance with changes in the experimental conditions. Choosing some points on either side of this will help to reduce the effect of noise in the spectroscopic data. However, if different species absorb in different parts of a spectrum, it may be very useful to include data from the different zones. We recommend that relatively few wavelengths are used for a HYPERQUAD calculation; there is a separate facility for the calculation of the molar absorbances at all wavelengths.

(iii) Trial and error fitting. This is similar to the facility in HydrapH. It works at a single wavelength, which is first chosen interactively. The procedure works as follows. The species concentrations are calculated from the model and the experimental conditions. These concentrations (c_j above) are then used to determine the molar absorbances at the chosen wavelength, from the Beer–Lambert law by weighted linear least squares. The weights are chosen as $1/(\text{absorbance})^2$ so as to minimize the sum of relative errors (another weighting scheme may be used in HYPERQUAD).

(iv) Calculation and display of the molar absorbances at all wavelengths. This calculation proceeds in the same manner as in (iii), but at all wavelengths. The calculated spectra may be stored in ASCII form for plotting by another program, such as EXCEL or ORIGIN. This calculation is a particularly useful check on the validity of a chemical model. Indeed, one would be loath to accept any model for which the calculated spectra of the absorbing species were unreasonable, as, for example, when they had many negative values.

(v) Factor analysis. Factor analysis was first introduced in this field by Kankare [10]. Since then, Zuberbühler and co-workers have developed the concept of Evolving Factor Analysis (EFA) [46,47], which they also describe as model-free analysis of spectrometric data. A similar approach, called "self-modelling curve resolution in studies of spectrometric titrations", has also been published [48]. There is no doubt that factor analysis has the potential to be very useful in studies of equilibria, but in practice it is not easy to apply.

The basis of the application of factor analysis is the Beer–Lambert law, implying that the total absorbance at any wavelength is a linear combination of the absorbances due to a few factors. When the number of (multi-wavelength) observations exceeds the number of factors, it is possible to smooth the data by back transformation using the correct number of abstract factors, as was done in CLINP [12]. However, it is not generally appreciated that this process is based on the

method of least squares. Indeed, the eigenvalues calculated in factor analysis are nothing other than the Langrangian multipliers of a constrained least-squares problem. Therefore, the application of factor analysis in equilibrium studies can be seen as another application of the method of least-squares.

We have used singular value decomposition of the matrix of absorbance values, A, to derive the eigenvalues and eigenvectors of A^TA. This procedure eliminates the accumulation of errors inherent in the matrix multiplication. A variety of statistics are provided for 1, 2, ..., eigenvalue models as suggested by Malinowski and Howery [49]. With the aid of these statistics, it should be possible to deduce the number of light-absorbing species present in the equilibria.

The difficulty with factor analysis is that the relationship between the eigenvalues and the experimental errors is an implicit one. It is therefore difficult to establish criteria to determine if a small eigenvalue is effectively non-zero. Conny and Meglen [50] have made an analysis of the effects of experimental noise on the calculated eigenvalues, but their conclusions are not very precise.

As far as Target Factor Analysis is concerned, we see no value in this technique, given that it is possible to perform a full equilibrium analysis. The idea is as follows. Suppose that there are n species to be considered. Then the first n eigenvectors are the abstract factors. To convert them into molar absorbances, a large rotation matrix must be found. The rotation matrix has too many variables to be determined correctly, but some progress can be made by, for example, imposing the condition that molar absorbance must be positive. This will establish ranges for the molar absorbances. The technique was first described in 1974 [51].

By contrast, EFA may be useful as long as one remembers that one is dealing with abstract fators. A careful reading of the original papers [46,47] will reveal that key words such as "species" and "concentration profiles" are written in quotation marks, as here.

2.9. The program Hyphen

This program is different from the other programs in the HYPERQUAD suite in that it does not require experimental data. It can be used to simulate titration curves or to give species distribution diagrams for a wide variety of conditions. Thus, it is a speciation program limited to homogeneous equilibria in solution. There is a large literature referring to speciation programs which we feel is inappropriate to be discussed here. Suffice it to say that the core of the program is the solution of the equations of mass balance by the Newton–Raphson method. This is a sub-problem as far as HYPERQUAD is concerned, so we use the same routines in Hyphen as are used in HYPERQUAD. The model used for these calculations is in a PAR file, but the conditions are stored separately in scratch files that serve merely to allow one to start again where one left off before.

In addition, Hyphen has the facility to calculate the species' concentrations at a single data point defined by knowing, for each reagent, either the total concentration or the free concentration (e.g. pH).

2.10. The program HYPERQUAD

As far as potentiometric data are concerned, the algorithm used in the HYPERQUAD is virtually the same as in SUPERQUAD [21]. Thus the new mathematical features are concerned with the treatment of spectrophotometric data or with the simultaneous treatment of both potentiometric and spectrophotometric data. There are significant differences between the present version of HYPERQUAD and that described in a preliminary account [34]. These arose out of the need to apply a rigorous weighting scheme to the data. The following outline is presented in terms of a relatively simple system of potentiometric measurements using an electrode responding to the hydrogen ion and absorbance measurement at nl wavelengths; there are three reagents, M, L and H, and nk equilibrium constants.

The objective function is given in matrix notation simply as $U = \boldsymbol{r}^T\boldsymbol{W}\boldsymbol{r}$, where $\boldsymbol{r}$ is a vector of residuals, $\boldsymbol{r} = (\boldsymbol{y}^{\text{observed}} - \boldsymbol{y}^{\text{calculated}})$, y^{observed} repre-

sents a measurment in mV, pH or absorbance and $\boldsymbol{W}$ is a matrix of weights. The residuals are ordered with potentiometric first and absorbance second. To minimize the objective function, we use the Gauss–Newton–Marquardt method summarized by the system of normal equations

$$(\boldsymbol{J}^T\boldsymbol{W}\boldsymbol{J} + \lambda\boldsymbol{D})\Delta\boldsymbol{p} = \boldsymbol{J}^T\boldsymbol{W}\boldsymbol{r}$$

where $\boldsymbol{J}$ is the Jacobian matrix and $\Delta\boldsymbol{p}$ is a vector of shifts to be applied to the parameters. $\boldsymbol{D}$ is taken as equal to the diagonal elements of $\boldsymbol{J}^T\boldsymbol{W}\boldsymbol{J}$ and λ is the Marquardt parameter which may, of course, be zero. The method requires that the parameters be given initial values; these may be obtained with the aid of HydrapH or HydraSP.

The elements of the Jacobian relative to any unknown parameter p are obtained from the defining equations, the modified Nernst law (ionic charges are omitted for simplicity of notation):

$$E = E^0 + f\frac{RT}{nF}\ln[\mathrm{H}]$$

$$\frac{\partial E}{\partial p} = \frac{\partial E}{\partial [H]}\frac{\partial [H]}{\partial p}$$

and the Beer–Lambert law:

$$A_\lambda = l\sum_{j=1,na}\varepsilon_{\lambda j}c_j$$

$$\frac{\partial A_\lambda}{\partial x} = l\sum_j \varepsilon_{\lambda j}\frac{\partial c_j}{\partial x}$$

$$\begin{array}{cccccccc}
0 & \cdots & 0 & 0 & \cdots & 0 & \cdots & 0 \\
\dfrac{\partial A_1}{\partial \varepsilon_{1,1}} & \cdots & \dfrac{\partial A_1}{\partial \varepsilon_{1,na}} & 0 & \cdots & 0 & \cdots & 0 \\
0 & \cdots & 0 & \dfrac{\partial A_2}{\partial \varepsilon_{2,1}} & \cdots & \dfrac{\partial A_2}{\partial \varepsilon 2_{,na}} & \cdots & 0 \\
\vdots & \cdots & \vdots & \vdots & \cdots & \vdots & \ddots & \vdots \\
0 & \cdots & 0 & 0 & \cdots & 0 & \cdots & \dfrac{\partial A_{nl}}{\partial \varepsilon_{nl,1}}
\end{array}$$

$$\begin{array}{ccccccccc}
\cdots & 0 & \dfrac{\partial E}{\partial \beta_1} & \cdots & \dfrac{\partial E}{\partial \beta_{nk}} & \dfrac{\partial E}{\partial \alpha_1} & \cdots & \dfrac{\partial E}{\partial \alpha_{nd}} \\
\cdots & 0 & \dfrac{\partial A_1}{\partial \beta_1} & \cdots & \dfrac{\partial A_1}{\partial \beta_{nk}} & \dfrac{\partial A_1}{\partial \alpha_1} & \cdots & \dfrac{\partial A_1}{\partial \alpha_{nd}} \\
\cdots & 0 & \dfrac{\partial A_2}{\partial \beta_1} & \cdots & \dfrac{\partial A_2}{\partial \beta \mathrm{nk}} & \dfrac{\partial A_2}{\alpha A_1} & \cdots & \dfrac{\partial A_2}{\partial \alpha_{nd}} \\
\cdots & 0 & \vdots & \cdots & \vdots & \vdots & \cdots & \dot{\partial} A_{nl} \\
\cdots & \dfrac{\partial A_{nl}}{\partial \epsilon_{nl,na}} & \dfrac{\partial A_{nl}}{\partial \beta_1} & \cdots & \dfrac{\partial A_{nl}}{\partial \beta_{nk}} & \dfrac{\partial A_{nl}}{\partial \alpha_1} & \cdots & \partial \alpha_{nd}
\end{array}$$

In addition, the system is subject to the constraint that the equations of mass balance are satisfied.

$$T_\mathrm{M} = [\mathrm{M}] + \sum_{k=1,nk} p_k\beta_k[\mathrm{M}]^{p_k}[\mathrm{L}]^{q_k}[\mathrm{H}]^{r_k}$$

$$= [\mathrm{M}] + \sum p_k c_k$$

is the equation for reagent M; similar equations may be written for L and H. Moreover, the total concentration T_M is obtained from the initial amount n_M, burette concentration a_M, initial volume v_0 and added volume v. Thus the three mass-balance equations are

$$\frac{n_\mathrm{M} + va_\mathrm{M}}{v_0 + v} = [\mathrm{M}] + \sum_{k=1,kn} p_k c_k$$

$$\frac{n_\mathrm{L} + va_\mathrm{L}}{v_0 + v} = [\mathrm{L}] + \sum_{k=1,nk} q_k c_k$$

$$\frac{n_\mathrm{H} + va_\mathrm{H}}{v_0 + v} = [\mathrm{H}] + \sum_{k=1,nk} r_k c_k$$

These are solved by the Newton–Raphson method and the normal equations matrix is used to derive $\partial[\mathrm{H}]/\partial\beta_k$ as described earlier [34]. Other Jacobian elements such as $\partial E/\partial E^0$, $\partial E/\partial n$ and $\partial E/\partial a$ are easy to derive. When quantities such as E^0 are refined they are termed dangerous parameters. Now, with these equations the Jacobian for one data point can be constructed, as in the following example applicable to a system with one electrode, *na* absorbing species, *nl* wavelengths, *nk* equilibrium constants and *nd* dangerous parameters, denoted by $\alpha_1, \ldots, \alpha_{nd}$. Note that we further assume here that there is a molar absorbance for each species at every wavelength.

At this point the normal equations matrix $\boldsymbol{J}^T\boldsymbol{W}\boldsymbol{J}$ and right-hand side $\boldsymbol{J}^T\boldsymbol{W}\boldsymbol{r}$ can be built up by accumulation. To do this we use the factored weight matrix, $\boldsymbol{W} = \boldsymbol{w}^T\boldsymbol{w}$. First the Jacobian and residual are left-multiplied by the factor and then the normal equations are accumulated as

$(wJ)^T(wJ)$ and $(wJ)^Twr$. This process destroys the block-diagonal structure[1] that was previously described for the normal equations matrix [34]. The normal equations that result have the dimensions of $na \times nl + nk + nd$. It is obvious that the number of wavelengths, nl, should not be large, otherwise the normal equations become large and hence slow and difficult to solve.

Solution of the normal equations provides shifts for all the parametes. However, the shifts on the molar absorbances are not used. Instead, the equilibrium constants and dangerous parameters are updated with the shifts and the molar absorbances are calculated using the updated values by linear least-squares applied to the Beer–Lambert law. In this procedure we follow what appears to be standard practice going back to LETAGROP–SPEFO [6]. However, some authors have applied a non-negativity constraint to the molar absorbances (SPECA [19], STAR [52]). We have not done this because we believe that a negative molar absorbance is a good indicator that something is wrong with the chemistry.

To understand how a negative molar absorbance, $\varepsilon_{\lambda j}$, can arise we must write out the Beer-Lambert law in full:

$$A_\lambda = l \sum_{j=1,na} \varepsilon_{\lambda j}\beta_j[\mathrm{M}]^{p_j}[\mathrm{L}]^{q_j}[\mathrm{H}]^{r_j}$$

Since the molar absorbance is multiplied by the equilibrium constant, this expression shows that we can expect the molar absorbances to be highly correlated with the equilibrium constants so that a small error in the latter can easily lead to a negative value in the former. Thus, negative molar absorbances indicate that the calculated equilibrium constants are in error.

The high correlation between molar absorbances and equilibrium constants is masked, but not eliminated, by the two-stage refinement process. It is a central problem in the determination of equilibrium constants from spectrophotometric data that can only be resolved by good experimental design. The calculation of all molar absorbances with the aid of HydraSP may reveal negative values at wavelengths not used in a HYPERQUAD calculation, so that it would be good practice to calculate all molar absorbances as a check on the results of a calculation based on selected wavelengths.

[1] The normal equation will have the block-diagonal structure if a diagonal weight matrix is used, but no special provision is made for this eventuality.

2.11. Calculation of the free concentrations

The equations of mass balance can be written in the most general form as $(i = 1, nr)$

$$T_i = [\mathrm{X}_i] + \sum_{k=1,nk} q_{ik}\beta_k \prod_{j=1,nr} [\mathrm{X}_j]^{q_{jk}}$$

where $[\mathrm{X}_i]$ represents the free concentration of the ith reagent. When the equilibrium constants, β, are "known", we have a system of nr non-linear simultaneous equations in nr unknown free concentrations. When these equations have been solved, the concentrations of all the species present in the equilibria will be "known". The solution of these equations is therefore required whenever we require the concentrations of the species, in HYPERQUAD, Hyphen, HydrapH and HydraSP. The calculation proceeds in two stages. First estimates must be made of all the free concentrations. Then these estimates are refined by Newton–Raphson iteration. Finally, the refinement must be terminated with a suitable criterion.

At the first point in a titration curve, or with batch data, we obtain first estimates by use of a modified Newton–Raphson technique with damping as suggested in the program ES4EC [53]. The modification is drastic: we set all off-diagonal elements of the Jacobian to zero, and improve each free concentration in turn. Subsequent points in a titration curve can utilise the last values calculated for the initial estimate. In the HYPERQUAD calculation some improvement in the initial estimate can be made on the second and subsequent iteration cycles. To illustrate this, consider the free hydrogen ion concentration at the jth point in a titration curve at the kth iteration cycle, ${}^k[\mathrm{H}]_j$. A better estimate than ${}^k[\mathrm{H}]_{j-1}$ (the concentration at the previous point in the titration curve) of this quantity is given by postulating that the slope relating to the free concentration is the same in the two iteration cycles, i.e. ${}^k[\mathrm{H}]_j -$

${}^{k}[\mathrm{H}]_{j-1} = {}^{k-1}[\mathrm{H}]_{j} - {}^{k-1}[\mathrm{H}]_{j-1}$. The time saved by improving the initial estimates of free concentrations is worthwhile as this calculation is the most time-consuming part of a HYPERQUAD calculation.

When it is known that the data points are equally spaced, as in Hyphen, the calculation can be speeded up by a simple extrapolation technique [54]. We have found that quadratic extrapolation works very well: $[\mathrm{X}]_i = [\mathrm{X}]_{i-3} - 3[\mathrm{X}]_{i-2} + 3[\mathrm{X}]_{i-1}$.

A serious problem can arise during a refinement if one or more of the equilibrium constants becomes negative. We allow this to happen as experience has shown that the "offending" constant may return to being positive later in the refinement. However, when an equilibrium constant is negative, there is no guarantee that all the free concentrations can be positive. (The condition is guaranteed by the existence of a unique free energy minimum at equilibrium when all the equilibrium constants are positive.) Now, we impose a non-negativity constraint on the free concentrations, as do most others, if for no other reason that we wish to take logarithms of free concentrations. Therefore, it can happen that the free concentration calculation may fail to converge. When this happens, we return to the main minimization routine and reduce the length of the shift vector for the equilibrium constants.

We attempt to satisfy the conditions of mass balance as nearly as is possible within machine accuracy. As double precision arithmetic is used throughout this means that the free concentrations are calculated to a relative precision of better than 1 part in 10^{15}.

2.12. Calculation of weights

In the "automatic" weighting scheme, the calculation of weights requires estimates of the errors on e.m.f., volume and absorbance. The last-named are derived from the empirical error function determined by the program HABER. Then, still assuming one electrode and *nl* wavelengths and assuming that the potentiometric and absorbnce data derive from a titration curve the variance–covariance matrix, $\boldsymbol{M}$, for a single point is given by the "rigorous" method of least squares to be [4].

	E	A_1	A_2	$\cdots$	A_{nl}
E	$s_E^2 + \left(\frac{\partial E}{\partial v}\right)^2 s_v^2$	$\left(\frac{\partial E}{\partial v}\right)\left(\frac{\partial A_1}{\partial v}\right)s_v^2$	$\left(\frac{\partial E}{\partial v}\right)\left(\frac{\partial A_2}{\partial v}\right)s_v^2$	$\cdots$	$\left(\frac{\partial E}{\partial v}\right)\left(\frac{\partial A_{nl}}{\partial v}\right)s_v^2$
A_1		$s_{A_1}^2 + \left(\frac{\partial A_1}{\partial v}\right)^2 s_v^2$	$\left(\frac{\partial A_1}{\partial v}\right)\left(\frac{\partial A_2}{\partial v}\right)s_v^2$	$\cdots$	$\left(\frac{\partial A_1}{\partial v}\right)\left(\frac{\partial A_{nl}}{\partial v}\right)s_v^2$
A_2			$sA_2^2 + \left(\frac{\partial A_2}{\partial v}\right)^2 s_v^2$	$\cdots$	$\left(\frac{\partial A_2}{\partial v}\right)\left(\frac{\partial A_{nl}}{\partial v}\right)s_v^2$
$\vdots$	symmetrical			$\vdots$	$\vdots$
A_{nl}					$s_{A_{nl}}^2 + \left(\frac{\partial A_{nl}}{\partial v}\right)^2 s_v^2$

The parital derivatives $\partial E/\partial v$ and $\partial A/\partial v$ required for the above formula are estimated from the observed data by means of piece-wise fitting of a seven-point cubic polynomial (simpler estimates are used for the first and last three points). We note that in SIRKO [14] the derivatives are obtained from the calculated values by analytical differentiation, but the authors remark that the weight should then be recalculated at every refinement cycle. The weight matrix, $\boldsymbol{W}$, would be the inverse of $\boldsymbol{M}$, but it is not actually calculated. Instead we perform a Choleski factorisation, $\boldsymbol{M} = \boldsymbol{m}^T\boldsymbol{m}$ and invert the lower-triangular factor, $\boldsymbol{m}$, to obtain the weight matrix factor, $\boldsymbol{w}$, such that $\boldsymbol{W} = \boldsymbol{w}^T\boldsymbol{w}$. The lower triangle of this factored matrix is stored in a single-precision one-dimensional array in order to conserve computer mem-

ory. Other weighting schemes are available. Diagonal weights may be used if the automatic scheme requires too much memory. Diagonal weights would be invoked automatically with batch as opposed to titration data.

For spectrophotometric data the alternative function

$$U = \sum_k \left(\frac{\Delta A_k}{A_k^{\text{obs}}} \right)^2; \quad \Delta A_k = A_k^{\text{calc}} - A_k^{\text{obs}}$$

may be minimized by means of the use of another weighting scheme. To see how this comes about, we derive the ith unit-weighted least-squares normal equation in the usual manner [4] as

$$\sum_k \sum_j J_{ki} J_{kj} \Delta p_j = \sum_k J_{ki} \frac{\Delta A_k}{A_k^{\text{obs}}}$$

The elements of the Jacobian, $\boldsymbol{J}$, are given by

$$J_{ki} = \frac{1}{A_k^{\text{obs}}} \frac{\partial A_k^{\text{calc}}}{\partial p_i}$$

where p_i is a refinable parameter. When this expression is substituted into the normal equation we obtain

$$\sum_k \sum_j \frac{\partial A_k^{\text{calc}}}{\partial p_i} \frac{\partial A_k^{\text{calc}}}{\partial p_j} \left(\frac{1}{A_k^{\text{obs}}} \right)^2 \Delta p_j = \sum_k \frac{\partial A_k^{\text{calc}}}{\partial p_i} \left(\frac{1}{A_k^{\text{obs}}} \right)^2 \Delta A_k$$

Thus, setting the weights as $W_k = (1/A_k^{\text{obs}})^2$ and minimizing the sum of squared residuals is equivalent to minimizing the sum of squared relative residuals with unit weights. However, when this weighting scheme is used it is not possible to give an expectation value for U.

2.13. The program Handout

The HYPERQUAD program produces a standard file, OUTPUT.RES, which contains all the useful information that was generated during the equilibrium constant refinement(s). The program HANDOUT has four main functions. Two of these relate to viewing the results on screen or dumping selected items to a printer. The third allows the model file to be updated with the refined values for the equilibrium constants. Lastly, there is a facility for drawing species distribution diagrams. These diagrams, which perforce relate to the experimental conditions, can be annotated by an interactive process involving the mouse, and the final diagram sent to a file. In that case, Hewlett-Packard (HP) Graphics Language is used so that the file may be dumped to an HP-compatible plotter or laser printer or imported into Word or WordPerfect as a picture.

2.14. Programming details

All programs were written in FORTRAN and compiled with MS FORTRAN 5.0 or 5.1 for an AT-style PC. They also appear to function correctly in a DOS window under Windows 95. They assume the presence of a Microsoft-compatible mouse, VGA graphics and a maths coprocessor. For HYPERQUAD there are no formal restrictions on the data. All arrays are dimensioned at run-time. This is achieved by first making a dummy pass through the data files to count all the quantities needed for the allocatable arrays. The arrays are then allocated and the data are read in. A similar system is used in HEDIT and most of the other programs. Thus, the only restriction on the data is imposed by the amount of memory available. It is our intention in the near future to recompile the programs with a 32-bit compiler; that will effectively remove all restrictions on data. Some restrictions may still remain that are imposed by what can be placed on the screen at the same time, but important screens, such as the one that shows the equilibrium constants, are already paged.

HYPERQUAD has been on beta release (mostly in Italy) for more than a year. All programs are fully documented. It has to be stated that we have encountered occasional difficulties with installation that we suspect are due to the use of non-standard mouse and keyboard routines (non-standard only in the sense that the routines are not in the compiler library).

3. Discussion

HYPERQUAD is different from all programs

that have been written before, in this field, in that it is not a single program but a suite of inter-related programs. This has been made possible by the developement of an associated data structure so that the HYPERQUAD programs access a common set of data files. By dividing the data conceptually into data relating to a model and data relating to experimental measurements of different kinds, it becomes possible to perform operations that were not possible before. For example, the model file can be used alone in the program Hyphen to simulate titration curves, that is, to find good experimental conditions, and then together with experimental data in the HYPERQUAD calculation. It is also possible that a model file which was designed for use with the program HypNMR [36] can be used in the simulation program Hyphen.

Within the suite there are many novelties. An important one is the idea of deriving an empirical function to relate the error on a spectrophotometric absorbance measurement to the absorbance value (program Haber). This function permits the construction of an approximate variance–covariance matrix for absorbances without the need to estimate the error on each absorbance measurement. That matrix is essential when it comes to determining the weights to be given to experimental observations in the least-squares calculation, particularly so when the observations may be of different kinds such as potentiometric and spectrophotometric.

Other new features are the creation of plot files in Hewlett-Packard Graphics Language for export to a plotter or laser printer, the use B-spline functions to locate end-points in potentiometric data, an offset procedure for coping with baseline errors in spectra and trial and error procedures for manual fitting of both potentiometric and absorbance data.

In summary, HYPERQUAD provides facilities, in one coherent package, for doing almost all processing of solution equilibrium data that has been done in the past, using standard data files and standard procedures for recalling data from files, for editing data and for saving data to files. The greatest novelty of the system is its comprehensive nature, with each program taking as input a standard model file and/or an experimental data file. The main difference between SUPERQUAD and HYPERQUAD is that the new program can handle spectrophotometric data in addition to potentiometric data.

4. Future developments

We do not believe that it is sensible for us to develop a program, such as SIRKO [14] or MICMAC [31], which claims to be able to handle any kind of data. Our experience has been that each kind of experimental data imposes certain requirements that are not present with the other. Thus, we are not attempting to integrate HypNMR [36] with HYPERQUAD because the NMR data usually consist of just single data points whereas HYPERQUAD is oriented towards data from titration curves. Likewise, we have a program PHAB already in an advanced stage of development as a separate entity. PHAB will deal with absorbance data at measured pH values so, unlike HYPERQUAD, the total hydrogen ion concentration is not known and the mass balance in protons is irrelevant. This imposes the need to calculate the free concentrations in a different way. The HYPERQUAD programs are highly modular so it is relatively easy to construct a new program for each substantially different kind of data. In addition, the user interface is standardized because it is based on a set of some 70 routines that constitute a HYPERQUAD subroutine library.

On the programming side, we must consider developing a Windows version of the software. The main advantages will be twofold. On the one hand we will be able to eliminate all restrictions on experimental data. At present the restrictions arise from the fact that we are limited to 640 k bytes of memory for program and data; that restriction disappears under Windows or with the use of a 32-bit compiler. The program which suffers most from this restriction is HydraSP, which can take a maximum of 57 344 data points (up to 28 spectra, each spectrum containing 2048 points). Second, there are restrictions imposed by what can be seen on the screen at any one time.

These restrictions can be removed by using automatically scrolling windows.

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References

[1] R.J. Motekaitis and A.E. Martell, The Determination and Use of Stability Constants, VCH, New York, 1988.
[2] D.J. Leggett (Ed.), Computational Methods for the Determination of Formation Constants, Plenum Press, New York, 1985.
[3] M. Meloun, J. Havel and E. Högfeldt, Computation of Solution Equilibria, Ellis Horwood, Chichester, 1994.
[4] P. Gans, Data Fitting in the Chemical Sciences, Wiley, Chichester, 1992.
[5] I. Nagypal, I Páka and L. Zekany, Talanta, 25 (1978) 549.
[6] L.G. Sillén and B. Warnqvist, Ark. Kemi, 31 (1968) 377.
[7] L.G. Sillén, Acta Chem. Scand., 18 (1964) 1085.
[8] J. Havel and M. Meloun, in D.J. Leggett (Ed.), Computational Methods for the Determination of Formation Constants, Plenum Press, New York, 1985, p. 221.
[9] P. Gans and A. Vacca, Talanta, 21 (1974) 45.
[10] J.K. Kankare, Anal. Chem., 42 (1970) 1322.
[11] L. Lampugnani, L. Meites, P. Papoff and T. Rotunno, Anal. Chim. Acta, 1994 (1987) 77.
[12] A.A. Bugaevskii, Yu. V. Kholin and D.S. Konyaev, Russ. J. Inorg. Chem., 38 (1993) 328.
[13] H. Gampp, M. Maeder and A. Zuberbühler, Talanta, 27 (1980) 1037.
[14] V.I. Vetrogon, N.G. Lukyanenko, M.-J. Schwing-Weill and F. Arnaud-Neu, Talanta, 41 (1994) 2105.
[15] T. Michalowski, Talanta, 39 (1992) 1127.
[16] M. Meloun, M. Javúrek, and J. Militký, Mikrochim. Acta, 109 (1992) 221.
[17] F. Gaizer and I.I. Kiss, Talanta, 41 (1994) 419.
[18] J.P. Chandler, R.E. Thomson, H.O. Spivey and E.L.-F. Li, Anal. Chim. Acta, 162 (1984) 399.
[19] R. Cazallas, M.J. Citores, N. Etxebarria, L.A. Fernández and J.M. Madariaga, Talanta, 41 (1994) 1637.
[20] C. De Stefano, P. Mineo, C. Rigano and S. Sammartano, Ann. Chim., 83 (1993) 243.
[21] P. Gans, A. Sabatini and A. Vacca, J.Chem. Soc., Dalton Trans., (1985) 1195.
[22] J. Havel and M. Meloun, Talanta, 33 (1986) 525.
[23] M. Meloun, M. Javúrek and J. Havel., Talanta, 35 (1986) 513.
[24] E. Bottari, T. Coccitto, G. Curzio, M.R. Festa and R. Jasionowska, Ann. Chim., 78 (1988) 635.
[25] V. Gadiokov and Kh. Mikhailova, Comput. Chem., 13 (1989) 325.
[26] Y.L. Sidrak and A. Aboul-Seoud, J. Comput. Chem., 8 (1987) 575.
[27] R.J. Motekaitis and A.E. Martell, Can. J. Chem., 60 (1982) 3403.
[28] A. Johansson and S. Johansson, Analyst, 104 (1979) 601.
[29] W.E. Gordon, Anal. Chem., 54 (1982) 1595.
[30] R.J. Motekaitis and A.E. Martell, Can. J. Chem., 60 (1982) 168.
[31] A. Laouenen and E. Suet, Talanta, 32 (1985) 245.
[32] A. Sabatini, A. Vacca and P. Gans, Talanta, 21 (1974) 53.
[33] P. Gans, A. Sabatini and A. Vacca, Inorg. Chim. Acta., 18 (1976) 239.
[34] A. Sabatini, A. Vacca and P. Gans, Coord. Chem. Rev., 120 (1992) 389.
[35] P.M. May and D.R. Williams, in D.J. Leggett (Ed.), Computational Methods for Determination of Formation Constants, Plenum Press, New York, 1985, p. 37.
[36] C. Frassineti, S. Ghelli, P. Gans, A. Sabatini, M.S. Moruzzi and A. Vacca, Anal. Biochem., 231 (1995) 374.
[37] R.S. McDonald and P.A. Wilks, Appl. Spectrosc., 42 (1988) 151.
[38] J.G. Grasselli, Pure Appl. Chem., 63 (1991) 1782.
[39] P.M. May, R. Murray and D.R. Williams, Talanta, 32 (1985) 485.
[40] A. De Robertis, C. De Stefano, S. Sammartano and C. Rigano, Anal. Chim. Acta, 222 (1989) 385.
[41] G. Gran, Analyst, 77 (1952) 661.
[42] F.J.C. Rossotti, and H. Rossotti, J. Chem. Educ., 42 (1965) 375.
[43] P. Gans and J.B. Gill, Appl. Spectrosc., 38 (1984) 370.
[44] C. de Boor, A Practical Guide to Splines, Springer, New York, 1978.
[45] J.T. Bulmer and H.F. Shurvell, J. Phys. Chem., 77 (1973) 2085.
[46] H. Gampp, M. Maeder, C.J. Meyer and A. Zuberbühler, Talanta, 32 (1985) 1133.
[47] H. Gampp, M. Maeder, C.J. Meyer and A. Zuberbühler, Talanta, 33 (1986) 943.
[48] R. Tauler, E. Casassas and A. Izquierdo-Ridorsa, Anal. Chim. Acta, 248 (1991) 447.
[49] E.R. Malinowski and D.G. Howery, Factor Analysis in Chemistry, Robert E. Krieger, Malabar, FL, 1989.
[50] J.M. Conny and R.R. Meglen, Anal. Chem., 64 (1992) 2580.
[51] E.A. Sylvestre, W.H. Lawton and M.S. Maggio, Technometrics, 16 (1974) 353.
[52] J.L. Beltrán, R. Codony and M.D. Prat, Anal. Chim. Acta, 276 (1993) 441.
[53] A. De Robertis, C. De Stefano, C. Rigano and S. Sammartano, Anal. Chim. Acta, 191 (1986) 385.
[54] B.W. Darvell and V.W.-H. Leung, Talanta, 37 (1990) 413.
[55] G. Arena, E. Rizzarelli, S. Sammartano and C. Rigano, Talanta, 26 (1979) 1.

[56] M. Cromer-Morin, J.P. Scharff and R.P. Martin, Analusis, 10 (1982) 92.
[57] P. Decock and B. Sarker, Can. J. Chem., 65 (1987) 2798.
[58] A.G. Asuero, J.L. Jiminez-Trillo and M.J. Navas, Talanta, 33 (1986) 531.
[59] R.A. Alcock, F.R. Hartley and D.E. Rogers, J. Chem. Soc., Dalton Trans., 115 (1978).
[60] M. Meloun and J. Militký, Mikrochim. Acta, 112 (1993) 155.
[61] M.J. Hynes, J. Chem. Soc., Dalton Trans., 311 (1993).
[62] C. De Stefano, P. Princi, C. Rigano and S. Sammartano, Ann. Chim., 77 (1987) 643.
[63] S. Koch and G. Ackermann, Z. Chem., 29 (1989) 150.
[64] A. Poznajlo, J. Radioanal. Nucl. Chem., 134 (1989) 97.
[65] A. Izquierdo and J.L Beltrán, Anal. Chim. Acta, 181 (1986) 87.
[66] F. Gaizer and H.B. Silber, Acta Phys. Chem., 36 (1990) 28.
[67] F. Gaizer and A. Puskás, Talanta, 28 (1981) 925.
[68] M. Wozniac, J. Canonne and G. Nowogrocki, J. Chem. Soc., Dalton Trans., 2419 (1981).
[69] M. Wozniak and G. Nowogrocki, Talanta, 25 (1979) 633, 643.
[70] Y. Nievergelt, Analyst, 119 (1994) 145.
[71] P.D. Taylor, I.E.G. Morrison and R.C. Hider, Talanta, 35 (1988) 507.
[72] G. Papanastasiou and I. Zogas, Talanta, 42 (1995) 827.
[73] M.J. Collins, J. Chem. Educ., 63 (1986) 457.
[74] R. Fournaise and C. Pettifaux, Talanta, 34 (1987) 385.
[75] L. Zekany and I. Nagypal, in D.J. Leggett (Ed.), Computational Methods for the Determination of Formation Constants, Plenum Press, New York, 1985, p. 291.
[76] D.D. Perrin and H. Stunzi, in D.J. Leggett (Ed.), Computational Methods for the Determination of Formation Constants, Plenum Press, New York, 1985, p. 71.
[77] H. Gampp, M. Maeder, C.J. Mayer and A. Zuberbühler, Talanta, 32 (1985) 95, 257.
[78] D.J. Legget, in D.J. Leggett (Ed.), Computational Methods for the Determination of Formation Constants, Plenum Press, New York, 1985, p. 159.
[79] A. Avdeef, in D.J. Leggett (Ed.), Computational Methods for the Determination of Formation Constants, Plenum Press, New York, 1985, p. 355.
[80] A. Zuberbühler and T.A. Kaden, Talanta, 29 (1982) 201.
[81] W.E. Wentworth, W. Hirsch and E. Chen, J. Phys. Chem., 71 (1967) 218.