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MULTIPARAMETRIC CURVE FITTING—VI. MRFIT AND MRLET, COMPUTER PROGRAMS FOR ESTIMATION OF THE STABILITY CONSTANT OF THE PREDOMINANT M_pL_q COMPLEX AND THE LIGAND PURITY BY ANALYSIS OF PHOTOMETRIC TITRATION CURVES

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Summary—MRFIT and MRLET, two FORTRAN computer programs, can analyse a photometric mole-ratio curve (photometric titration curve) to estimate the stability constant β_{pq} of the predominant complex M_pL_q, the ligand concentration factor f_L, the extrapolated absorbance A_{ext} and the stoichiometric coefficient q (p is usually 1). MRFIT uses algorithmic minimization of a residual-square sum to reach, usually, the global minimum or the lowest of several local ones. MRLET, an ABLET system program based on LETAG, allows algorithmic and/or heuristic minimization. A local minimum described by parametric co-ordinates with a definite physical meaning might be found by the heuristic process.

The mole-ratio method introduced by Yoe and Jones[1,2] for spectrophotometric investigation of complex-formation equilibria has been frequently used,[3–9] and is known as photometric titration in analytical chemistry. It is based on graphical interpretation of the absorbances observed when the concentration of one component of the complex is varied while that of the other is held constant.

If the system forms a sufficiently stable complex, such a plot gives a sharp break and the mole ratio at the break corresponds to the composition of the complex. If, however, a weak complex is formed, only a curved plot results (Fig. 1). Previous papers in this area have been reviewed by Momoki *et al.*[9] who also suggested a new generalized approach to the mole-ratio method to allow computer-assisted treatment.

We introduce here two FORTRAN programs that use different mathematical approaches to the minimization of a residual-square sum function. The program MRFIT is easy to apply because its algorithmic strategy works completely automatically, and minimization always leads to the global minimum or to the lowest of several minima. The parametric co-ordinates of the minimum always have mathematical meaning, although in some cases they may have no physical one.

The program MRLET, part of the ABLET system,[10] allows application of an algorithmic strategy or a heurisitic strategy, or a combination of the two. This makes it possible to find a local minimum with parametric co-ordinates with definite physical meaning.[10] Heuristic minimization requires some intervention by the user, to set the initial size of the minimization steps, the initial guesses for the parameters to be estimated or the value of the parametric steps for the final refinement of parameters.

THEORY

Mathematical model—equation of a photometric titration curve

A photometric titration curve is a graphical representation of the mole-ratio method. It shows how the

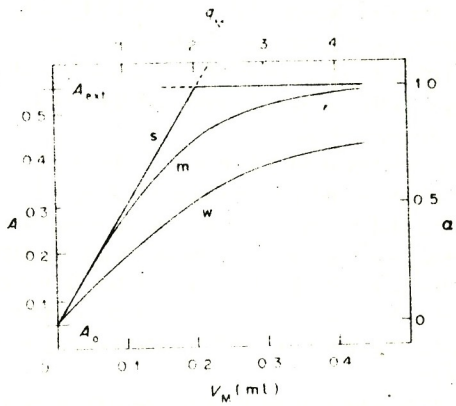

Fig. 1. Photometric mole-ratio curves shown in original (A; V_M) and normalized (α; q_M) co-ordinates for complex M_pL. Curves for strong (s), medium (m) and weak (w) complexes are shown.

Part V. *Talanta*, 1984, **31**, 947.

absorbance changes as the mole rátio of the two components M and L of the complex M_pL_q is changed when the concentration of one is varied and the concentration of the other is held constant. Let us consider the formation of a single complex M_pL_q by the reaction

$$pM + qL \rightleftharpoons M_pL_q \quad (1)$$

with (concentration) formation constant

$$\beta_{pq} = [M_pL_q]/([M]^p[L]^q) \quad (2)$$

$$\beta_{pq} = [M_pL_q]/\{(c_M - p[M_pL_q])^p(c_L - q[M_pL_q])^q\} \quad (3)$$

If the total concentrations of M and L are denoted by c_M and c_L, the mass-balance equations are

$$c_M = [M] + p[M_pL_q] = c_{M,b}V_M/V_0 \quad (4)$$

$$c_L = [L] + q[M_pL_q] = c_{L,w}f_L \quad (5)$$

where $c_{M,b}$ is the molar concentration and V_M the volume of the metal-ion solution added from the burette, V_0 is the initial titrand volume, $c_{L,w}$ is the nominal ligand concentration, calculated from the weight of reagent used and volume of solution made from it, and f_L is a concentration factor to allow for impurity in the reagent.

The mole ratio is $q_M = c_M/c_L$ and the normalized absorbance α is defined by the expression[9]

$$\alpha = [A(1 + V_M/V_0) - A_0]/(A_{ext} - A_0) \quad (6)$$

where A_0 is the initial absorbance and A_{ext} is the absorbance of a solution in which an excess of metal ion has been added to suppress dissociation of the complex. For constant ligand concentration the following function may be derived:

$$F = \alpha p/q + \alpha^{1/p}/[q\beta_{pq}c_L^{(p+q-1)/p}(1-\alpha)^{p/q}] - q_M = 0 \quad (7)$$

This describes the experimental relation $A = f(q_M)$ or the photometric titration curve $A = f(V_M)$. This mathematical model contains the dependent variable (A), the independent variable (V_M), and three unknown parameters, the overall stability constant (β_{pq}), the absorbance of the complex M_pL_q (A_{ext}), and the ligand concentration factor (f_L). The two stoichiometric coefficients of the complex, p and q, are usually suggested by the user and then validated by calculation; p is usually 1.

The relationship between absorbance and mole ratio is measured for constant total ligand concentration, and varied metal ion concentration, so the method is known as the "metal-changing method" (MCh). When the ligand concentration is varied and the metal-ion concentration is kept constant, the method is known as the "ligand-changing method" (LCh).[9]

If only one complex is formed (and it is very stable), the graph of $A = f(q_M)$ consists of two intersecting lines (Fig. 2). The ratio of the total concentrations of metal and ligand at the point of intersection gives the metal-to-ligand ratio in the complex. If the complex is only moderately stable, a steep curve is obtained and the intersection of tangents to the initial and final sections can still give this ratio, but the uncertainty increases with decreasing stability. For weak complexes, this simple graphical method fails to give any reliable information. If N complexes are formed and the successive formation constants are sufficiently high for there to be little overlap of the equilibria, the curve consists of $(N + 1)$ segments. If a single complex predominates, regression evaluation of the mole-ratio curve can be used to estimate the three parameters β_{pq}, f_L, and A_{ext}.

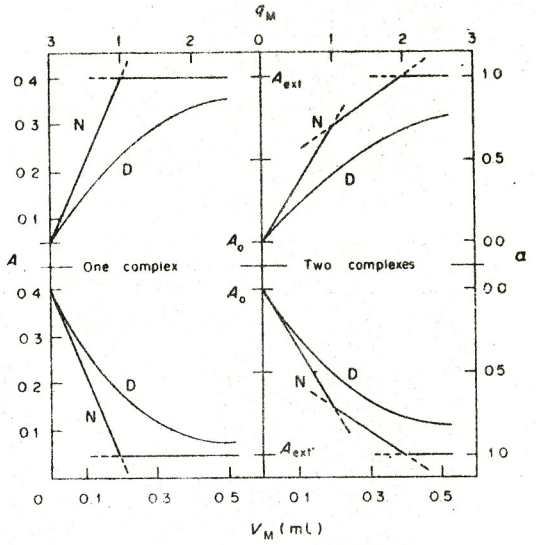

Fig. 2. Photometric mole-ratio curves shown in original (A; V_M) and normalized (α; q_M) co-ordinates for a single prevailing complex ML in solution (left half of figure) and a mixture of complexes ML and M_2L (right half), for strong (N) and weak (D) complexes in solution. The photometric titration curve is shown for the wavelength at which the complex absorbs (upper part) or at which the ligand absorbs (lower part).

Regression analysis

Regression analysis consists of the estimation of unknown parameters by minimizing the difference between experimental and calculated data. The theoretical justification for the procedure has recently been discussed.[10]

The experimental error in reading the burette is usually less than the error in the absorbance reading. Because an explicit expression of the absorbance $A = f(V_M)$ is not possible, the calculated absorbance A_{calc}, is obtained by the Müller approximation[11] in both MRLET and MRFIT. The sum of weighted squared residuals

$$U = \sum_{i=1}^{n} w_i(A_{exp,i} - A_{calc,i})^2 = \text{minimum} \quad (8)$$

where w_i is the statistical weight, usually taken as unity, can be minimized by use of the least-squares procedure LETAG[12] for MRLET, and FIT[13] for

MRFIT. The FIT subroutine uses only algorithmic minimization, and always finds the global minimum. The normal equations are linearized in FIT and transformed to the principal axis system. The subroutine contains a main loop for calculating alterations to parameters from the eigenvalues and eigenvectors of the normal equations, with reduction of the alterations if the linearity range is exceeded, and a minor loop for reducing the linearity ranges if it is found that the squared error obtained with the new parameters is greater than that for the old ones. The termination criterion is tested only in normal iterations.

The MRLET program, from the ABLET system,[10] uses the pit-mapping algorithm LETAG,[12] and does the minimization with heuristic (steps controlled by the user) or algorithmic (steps controlled automatically by the program) control. Heuristic minimization may reach a local minimum with definite physical meaning, but the algorithmic process always finds the global minimum or lowest minimum of several, but this does not necessarily have a physical meaning. In the heuristic process, the user must decide whether it is sufficient to know the location of some local minimum or whether knowledge of the global minimum is required. The user has to supply initial guesses of the values of the parameters and the minimization steps, and an organizational framework to control the process from iteration to iteration.

For all the parameters involved here, a negative value has no physical meaning. For such parameters the MRLET program contains a safeguard to stop them from becoming negative during the minimization, and at the same time to check that the calculations are not carried too far from the minimum. The block doing this is called MIKO; it searches systematically, over the calculated second-degree surface, for the set of three parameters that gives the lowest value for U with no parameter negative.

DATA INPUT INSTRUCTIONS

MRFIT

The data cards required for MRFIT are as follows, and each photometric titration requires this sequence of cards.

1. *Title card*: TITLE (20A4)
2. *Termination card*: STOP, SINST.
3. *Titration conditions card*: NB, CMB, VO, AL, CLW, CORM, ALFMIN, ALMAX, P, Q.
4. *Data card*: for simulated data set: VM(I), I = 1, NB; for experimental data set: VM(I), AEX(I), I = 1, NB.
5. *Initial guess card*: BETAO, FLO, AEXTO.
6. *Simulation card*: for a simulated data set: BETA, FL, AEXT; for an experimental data set this card is omitted.

The *termination card* contains STOP values (the algorithm FIT terminates when the sum of squares of parameter alterations divided by their variances is less than the STOP value), and SINST, the standard deviation of the absorbances measured with the spectrophotometer used: SINST > 0 indicates that a simulated data set is used, and SINST ≤ 0 that the experimental data are used.

The *titration conditions card* contains ten experimental quantities: NB is the number of data points, CMB is the molar concentration of metal solution in the burette, $c_{M,b}$, VO is the initial volume of titrand solution V_0, AL is the initial absorbance of the ligand in the titrand before the titration is started, CLW is the total molar concentration of the ligand in the titrand, $c_{L,w}$, CORM is a correction factor relating the burette volumes $V_{M,i,read}$ to true volumes delivered [so $V_{M,i}$ = VM(I)*CORM], ALFMIN is the lower limit α_{min} of the curve interval to be analysed numerically and ALFMAX is the upper limit α_{max}, and P and Q are stoichiometric coefficients p and q of the complex M_pL_q.

The *data card* contains experimental pairs of titration-curve points; VM(I) is the volume $V_{M,i,read}$ of metal solution added and AEX(I) is the measured absorbance (when NB is written with a minus sign, transmittance is read in instead of absorbance). With simulated data, only the independent variable VM(I) is read here, and the dependent variable, absorbance, is generated by MRFIT.

The *initial guess card* contains the initial guesses of the three parameters to estimated: $\beta_{pq}^{(0)}$, $f_L^{(0)}$, $A_{ext}^{(0)}$.

The *simulation card* contains preselected values BETA, FL, AEXT for the parameters β_{pq}, f_L, and A_{ext}. For experimental data, this card is omitted.

MRLET

Programs of the ABLET system all use similar data decks, as discussed previously.[10] Only the three specific cards (the *simulation card, titration conditions card* and *data card*) are commented on in detail here.

1. *Title card*: TITLE (20A4).
2. *Keys card*: ISSW(I), I = 1, 6.
3. *Termination card*: EPS, PSI, SINST.
4. *Simulation card*: for a simulated data set: (XK(I), I = 1, 4), (SIGXK(I), I = 1, 4), (WEI(I), I = 1, 4). For an experimental data set this card is omitted.
5. *Titration conditions card*: for a simulated data set: NB, CMB, VO, AL, CLW, CORM, ALFMIN, ALFMAX, P, Q, FLO, AEXTO; for an experimental data set: 6, plus the same deck as for a simulated data set.
6. *Data card*: for a simulated data set: VM(I), I = 1, NB; for an experimental data set: VM(I), AEX(I), I = 1, NB.
7. *Initial guess card*: 7, 4, 4, AEXTO, FLO, LBETAO, QO.
8. *Matrix card*: ISKIN, (I(K), J(K), S(I(K), J(K)), K = 1, ISKIN.
9. *Step card*: 3, N, (I(K), STEK(I(K)), K = 1, N).
10. *Process card*: IRUR, IRUR, IRUR, ... (e.g., 2, 5, 13).

This set of cards constitutes one data block, and as many data blocks as desired may be executed, one after the other. The *simulation card* reads preselected values of four parameters in sequence (A_{ext}, f_L, log β_{pq}, q) followed by their standard deviations $s(A_{ext})$, $s(f_L)$, $s(\log \beta_{pq})$, $s(q)$ and their weights $w(A_{ext})$, $w(f_L)$, $w(\log \beta_{pq})$, $w(q)$.

The titration conditions card contains the same quantities as for MRFIT. FLO and AEXTO here represent the initial guesses $f_L^{(0)}$, $A_{ext}^{(0)}$. For an experimental data set, this card must begin with the integer key 6.

The *data card* has the same content as for MRFIT.

The *initial guess card* contains the key 7 followed by the number of parameters to be refined and the number of positive parameters. Values of the initial guesses for particular parameters in the sequence $A_{ext}^{(0)}$, $f_L^{(0)}$, log $\beta_{pq}^{(0)}$, $q^{(0)}$ follow.

The remaining cards have been described previously.[12]

EXPERIMENTAL

Photometric titration techniques

The mole-ratio curve for a complex-forming system may be measured by two methods.

(1) *The MCh (metal-changing) method.* The concentration of the ligand in the titration vessel is kept constant

and this solution is titrated with the metal-ion solution added from a burette.

(2) *The LCh (ligand-changing) method.* The concentration of metal-ion solution in the titration vessel is kept constant and the amount of ligand is gradually increased by addition from a burette.

There are two distinct experimental techniques for doing photometric titrations.

(1) *Titration done in the cell (internal MCh technique).* A known volume of ligand solution (2–30 ml) is placed in the spectrophotometer cell. For the total increase in volume during the titration to be small enough for the ionic strength and some equilibrium properties not to be seriously affected, the volume of metal solution added should be in the range 100–300 μl. A stirrer and the capillary tip of the microburette are inserted in the cell (and a glass electrode if it is desired to check the pH during the titration). The geometric arrangement should be unchanged throughout the titration. If necessary, polyethylene inlet tubes are also inserted into the cap to allow the cell to be flushed with a solvent-saturated stream of inert gas.

(2) *Titration done outside the cell (external MCh technique).* A 150-ml double jacket titration vessel is connected to the photometer cell by polyethylene tubes or glass capillary tubes fitted with glass ball-joints. Tubes pass through the Teflon cell-stopper, one of them connecting the bottom of the cell to a 100-ml glass syringe. The titration of the ligand solution is done in this titration vessel, the reagents being mixed by passage of inert gas or by a mechanical stirrer. After each addition of metal ion solution and establishment of equilibrium, some solution is transported into the cell by overpressure of inert gas or by use of a syringe. The cell is rinsed several times with the solution from the titration vessel, the absorbances at various wavelengths are measured, and the solution is transported back into the vessel. The concentration of metal is then changed by adding solution from a microburette and the whole procedure is repeated.

APPLICATIONS

Some examples are given below of systems in which a predominant complex exists at equilibrium, and which were studied with the aid of MRFIT and MRLET.

Example 1. SNAZOXS–zinc complex, studied by the internal MCh technique.

The stability constant of the ML_2 complex and the ligand concentration factor are estimated by the MCh data-evaluation procedures of MRFIT and MRLET. The two minimization strategies, algorithmic and heuristic, are illustrated.

Experimental conditions. An initial volume of 4.00 ml (= VO) of $1.65 \times 10^{-4} M$ (= CLW) SNAZOXS, was titrated with $2.0 \times 10^{-3} M$ (= CMB) zinc added from a home-made microburette having a correction factor of 0.988 (= CORM). The titration was done in acetate buffer at pH 5.5, with $I = 0.1$ ($NaClO_4$), with measurement at 575 nm in a 1.000 cm cell at 25°; these experimental conditions are given in the title card. The absorbance before the titration was started was 0.670 (= AL).

Input data for MRFIT (Table 1). Program MRFIT will test the proposed chemical model and verify or reject the existence of complex ML_2, the input data being P = 1, Q = 2, and the initial guesses $\beta_{12}^{(0)} = 10^8$ (= BETAO), $f_L^{(0)} = 1.0$ (= FLO), and $A_{ext}^{(0)} = 0.01$ (= AEXTO). Selected points from the total 24 (= NB) points of the photometric titration curve, between $\alpha_{min} = 0.01$ (= ALFMIN) and $\alpha_{max} = 0.99$ (= ALFMAX) will be used. The experimental data are executed, so SINST must be equal to -1. The MRFIT algorithmic minimization is limited to 100 iterations (program code) or by the termination criterion 10^{-9} (= STOP) in the input.

Input data for MRLET (Tables 2 and 3). Program MRLET can perform an algorithmic (Table 2) or a heuristic (Table 3) minimization. In each iteration a print of the parameter values and the residual-square sum function is requested [ISSW(1) = 1] and also a print of the elements of the twist matrix [ISSW(2) = 1] and the determinant [ISSW(3) = 1]. When an algorithmic process is used, [ISSW(6) = 0], the minimization step in the next iteration is calculated either as a PSI fraction (PSI = 0.3) of the previous step [STEP(I) = PSI* STEP(I)], in which case ISSW(4) is equal to 0, or as a PSI fraction of the parametric standard deviation DARK2(I) calculated in the previous iteration [STEP(I) = PSI* DARK2(I)], in which case ISSW(4) is equal to 1. The key ISSW(5) is used for simulated data only. The minimization process is terminated when $|(U^{(i)} - U^{(i+1)})/U^{(i)}| \leq EPS$ (here $EPS = 10^{-6}$) or when 40 iterations have been performed. As experimental data are to be used, SINST is -1.0. When no twist matrix elements are available, ISKIN is equal to 0 and the rest of the card may be omitted. The step card contains IRUR = 3 which calls the block STEG,

Table 1. MRFIT input data for Example 1

Card	Content of card
1. Title card	ZN + SNAZOXS. PH = 5.5, I = 0.1,575 NM, 1.000 CM,
2. Termination card	1.0E-9, −1.0,
3. Titration conditions	24, 0.002, 4.0, 0.670, 1.65E-4 0.988, 0.01, 0.99, 1, 2,
4. Data card	0.0100, 0.628, 0.0296, 0.547, 0.0494, 0.468, 0.0692, 0.392, 0.0889, 0.318, 0.0988, 0.286, 0.1087, 0.255, 0.1136, 0.241, 0.1186, 0.227, 0.1235, 0.214, 0.1284, 0.202, 0.1334, 0.195, 0.1383, 0.182, 0.1433, 0.174, 0.1482, 0.164, 0.1531, 0.159, 0.1581, 0.151, 0.1680, 0.141, 0.1704, 0.138, 0.1754, 0.138, 0.1778, 0.139, 0.1877, 0.121, 0.1976, 0.117, 0.2075, 0.110,
5. Initial guess card	1.0E8, 1.0, 0.01,
6. Simulation card	For experimental data this card is omitted.

Table 2. MRLET input data for Example 1 (algorithmic minimization)

Card	Content of card
1. Title card	ZN + SNAZOXS, PH = 5.5, I = 0.1, 575 NM, 1.000 CM, ALGORITHMIC STRATEGY, MRLET
2. Keys card	1, 1, 1, 0, 1, 0,
3. Termination card	1.0E-6, 0.3, −1.0,
4. Titration conditions card	6, 24, 2.0E-3, 4.0, 0.670, 1.65E-4, 0.988,, 0.01, 0.99, 1, 2, 0.9, 0.01,
5. Data card	the same as Data card in Table 1.
6. Initial guess card	7, 4, 4, 0.01, 0.9, 9.0, 2.0,
7. Matrix card	0,
8. Step card	3, 3, 1, 0.04, 2, 0.01, 3, 0.2,
9. Process card	2, 5, 13,

Table 3. MRLET input data for Example 1 (heuristic minimization)

Card	Content of card
1. Title card	ZN + SNAZOXS, PH = 5.5, I = 0.1, 575 NM, 1.000 CM, HEURISTIC STRATEGY, MRLET
2. Keys card	1, 1, 1, 0, 1, 1,
3–7. The same as corresponding cards in Table 2.	
8. Step card + process card	3, 1, 3, 0.06, 2, 5,
9. Step card + process card	3, 3, 1, 0.04, 2, 0.01, 3, 0.2, 2, 5,
10. Final card	−1,

followed by the number of parameter steps ($N = 3$) and the number, I(K), and numerical value STEK(I(K)) of each parameter step. This STEK(I(K)) step value is used in the first iteration only; in following iterations it is calculated as indicated above. The process card lists three IRUR numbers (2, 5, 13) which call three blocks; 2 calls UTTAG, *i.e.*, the starting value of the first central point of the residual-square sum function; 5 calls LETA for systematic variation of parameters and the minimization process; 13 calls SKRIK for output of the results and fitness test.

When a heuristic minimization is required the key ISSW(6) is set equal to 1 (*cf.* Table 3), and minimization steps and process keys IRUR are read from data cards in each iteration. One iteration is executed after reading in IRUR = 5, which calls the block LETA. The parameters do not all need to be refined at once. They are estimated one at a time, then in the final iteration all are refined together. This heuristic strategy is recommended, for example, when there are some ill-conditioned parameters in the model or when the pit-shape is skew or plate-like.

Example 2. The 7-(2-carboxyphenylazo)-8-hydroxyquinoline-5-sulphonic acid–copper(II) complex studied by the external MCh technique.

The stability constant of ML and the ligand correction factor are estimated by MRFIT analysis of mole-ratio curves measured at various wavelengths.

Experimental conditions. An initial volume of 20.00 ml (= VO) of $1.102 \times 10^{-4}M$ (= CLW) ligand solution, was titrated with $3.39 \times 10^{-3}M$ (= CMB) copper(II) added from a home-made syringe microburette with a correction factor of 0.01947 ml/nmi (= CORM). The titration was done in acetate buffer at pH = 4.901, and $I = 0.1$ ($NaClO_4$) with measurement at 510 nm in a 1.000-cm cell at 25°. The initial absorbance was 1.210 (= AL).

Input data for MRFIT (Table 4). MRFIT will test the proposed chemical model ML, the parameters being P = 1, Q = 1, β_{11} = 10^6 (= BETAO), $f_L^{(0)} = 0.9$ (= FLO), $A_{ext}^{(0)} = 0.56$ (= AEXTO). Selected points from the total of 13 (= NB) on the photometric titration curve between $\alpha_{min} = 0.01$ (= ALFMIN) and $\alpha_{max} = 0.99$ (= ALFMAX) will be used for curve fitting. Because experimental data are used, SINST is

Table 4. MRFIT input data for Example 2

Card	Content of card
1. Title card	OB + CU, PH = 4.901, I = 0.1, 510 NM, 1.000 CM,
2. Termination card	1.0E-9, −1.0,
3. Titration conditions card	13, 3.39E-9, 20.0, 1.210, 1.102E-4, 0.01947, 0.01, 0.99, 1, 1,
4. Data card	2.22, 1.162,, 8.89, 1.019, 11.12, 0.971, 13.33, 0.924, 15.57, 0.878, 17.79, 0.831, 20.00, 0.785, 22.24, 0.738, 24.46, 0.694, 26.68, 0.652, 28.91, 0.620, 31.12, 0.592, 35.57, 0.559,
5. Initial guess card	1.0E6, 0.9, 0.56,
6. Simulation card	For experimental data this card is omitted.

Table 5. MRFIT input data for Example 3 (simulated data)

Card	Content of card
1. Title card	SIMULATED DATA SET, EXAMPLE 3,
2. Termination card	1.0E-9, 0.006,
3. Titration conditions card	28, 1.0E-3, 20.0, 0.0001, 1.0E-5, 1.000, 0.01, 0.99, 1, 1,
4. Data card	0.0222, 0.0355, 0.0450, 0.0569, 0.0686, 0.0808, 0.0934, 0.1064, 0.1200, 0.1344, 0.1405, 0.1500, 0.1533, 0.1566, 0.1601, 0.1636, 0.1671, 0.1708, 0.1746, 0.1785, 0.1825, 0.1866, 0.1910, 0.1954, 0.2001, 0.2049, 0.2100, 0.2153,
5. Initial guess card	1.5E6, 0.9, 0.95,
6. Simulation card	1.000E6, 1.0, 1.0,

-1.0. The MRFIT minimization is algorithmic, limited to 100 iterations or by the termination criterion 10^{-9} (= STOP).

Example 3. Data simulation. The influence of the instrumental error of the spectrophotometer used, $s_{inst}(A)$, on the estimated parameters can be investigated by the use of simulated data, by MRFIT.

Experimental conditions. For parameters $\beta_{11} = 10^6$, $f_L = 1.0$, $A_{ext} = 1.0$ and various preselected values of the instrumental error, *viz.* $s_{inst}(A) = 10^{-6}$, 10^{-4}, 10^{-3}, 0.002, 0.004, 0.006, (= SINST), and with the rest of the experimental conditions kept constant (NB = 28, CMB = 0.001, VO = 20.0, AL = 0.0001, CLW = 0.00001, CORM = 1.0, ALFMIN = 0.01, ALFMAX = 0.99, P = 1, Q = 1, BETAO = 1.5E6, FLO = 0.9, AEXTO = 0.95) six mole-ratio curves are generated. After data simulation, a MRFIT minimization is done. The input data for simulation and minimization process are given in Table 5.

DISCUSSION

Table 6 shows the MRFIT output for Example 1. The first part lists the experimental conditions and the calculation conditions. All 24 experimental points are used in the regression analysis because they lie between α_{min} and α_{max}. TITRATION DATA lists the original co-ordinates $\{V_{M,read}; A_{read}\}$ of the photometric titration points and SELECTED POINTS lists the transformed co-ordinates $\{c_M; A\}$, the ith point having the co-ordinates $c_{M,i} = c_{M,b} * V_{M,read,i} * CORM/V_0$ and $A_i = A_{i,read}(1 + V_{M,i,read} * CORM/V_0)$. MRFIT solves equation (7) by using equations (5) and (6) and the mole-ratio curve in transformed co-ordinates $\{c_M; A\}$.

INTERMEDIATE RESULTS gives the residual-square sum for the initial guesses of the parameters and for the fitted values found at the end of minimization.

OUTPUT lists the values of the parameters and their absolute and relative standard deviations. When the elements of the covariance matrix are negative, they are not printed, and the standard deviations of the parameters are also not defined. CURVE FITTING lists the experimental and calculated points of the photometric tritration curve in the original $\{c_M; A\}$ and normalized $\{q_M; \alpha\}$ co-ordinates. The residuals demonstrate the quality of the fit achieved. The degree of fit is tested objectively by the statistical analysis of the residuals.

MRFIT verified the proposed chemical model, that the ML_2 complex is formed in the photometric titration of zinc with SNAZOXS, when excess of ligand is present. The statistical tests show that the calculated and experimental points are very close: the mean value of the absorbance residuals is only 0.0012 (*i.e.*, the arithmetic mean of the absolute values of the residuals), less than the instrumental error for the spectrophotometer used [$s_{inst}(A) = 0.002$ for the Zeiss VSU2-G spectrophotometer], and the standard deviation 0.0020 of the residual mean is of the same magnitude as the instrumental error. The other statistical tests also prove that the degree of fit is sufficiently good for the parameter estimates found to be considered reliable.

The statistical test of the degree of fit used here is an efficient diagnostic tool when a chemical model is sought, and it may be used as a criterion for comparison and selection of the best model from several plausible proposed ones.

From the numerical analysis of the photometric titration of zinc with SNAZOXS, it may be concluded that for the range from excess of ligand in solution to nearly equimolar solutions ($q_L = c_L/c_M = 31.6$–1.54) the ML_2 complex prevails in solution. This complex has a stability constant $\beta_{12} = 3.05 \times 10^9$, the concentration factor of SNAZOXS is $f_L = 0.947$ (this means that the SNAZOXS has a purity of 94.7%) and the extrapolated absorbance is $A_{ext} = 0.01205$ (Table 6).

Table 8 shows a MRLET output for Example 1. Experimental conditions and curve-point selection are the same as for MRFIT. Most of the MRLET output is self-explanatory, but some comments are appropriate. The control label RURIK indicates the sequence of operations in the minimization process. RURIK = 6 introduces block DATA, which reads the titration conditions card and data card and prints their content in the output. RURIK = 7 calls block LASK which reads the initial guesses of the four parameters to be refined, and RURIK = 3 reads and writes the minimization steps for the first iteration. RURIK = 2 causes calculation of the residual-square sum U_c for the central point in the first iteration, *i.e.*, for the initial guesses of the parameters.

Table 6. Shortened MRFIT output for Example 1 (Table 1)

MRFIT PROGRAM

MRFIT-TITLE: ZN + SNAZOXS, PH = 5.5, I = 0.1,575 NM, 1.000 CM,
TYPE OF INPUT: EXPERIMENTAL DATA

TITRATION VESSEL: TOTAL LIGAND CONCENTRATION (MOL/L)	= 0.000165
INITIAL VOLUME (ML)	= 4.00
INITIAL ABSORBANCE	= 0.670
BURETTE: FACTOR OF MICROBURETTE (ML PER 1 MM)	= 0.988
METAL STOCK SOLUTION(MOL/L)	= 0.00200
CURVE FITTING: SEGMENT OF CURVE TAKEN TO REGRESSION	
FROM ALPHAMIN	= 0.0100
TO ALPHAMAX	= 0.9900
TESTED STOICHIOMETRY OF COMPLEX P = . . . , Q = . . . ,	1 2
NUMBER OF POINTS OF THE WHOLE TITRATION CURVE	= 24
NUMBER OF POINTS OF THE CURVE SEGMENT TAKEN TO REGRESSION	= 24
TERMINATION CRITERIA: MAXIMUM NUMBER OF ITERATIONS DECL.	= 100
PRECISION WHEN COVARIATION MATRIX IS CALCULATED	= 1.0E-9
INITIAL GUESS OF FIRST PARAMETER (STABILITY CONSTANT, K)	= 1.0E8
INITIAL GUESS OF SECOND PARAMETER (LIGAND FACTOR, FL)	= 1.000
INITIAL GUESS OF THIRD PARAMETER (ABSORBANCE EXTRAPOLATED)	= 0.010

INTERMEDIATE RESULTS:
IN 23 ITERATIONS THE NORMAL EQUATIONS WERE ILL CONDITIONED
IN 24 ITERATIONS THE CALCULATED ALTERATION OF PARAMETERS WAS GREATER THAN THE ESTIMATED LINEARITY RANGE
RESIDUAL-SQUARED SUM/DEGREES OF FREEDOM WITH INITIAL GUESS: 4.283E-02 AND WITH FITTED PARAMETERS: 4.684E-06

OUTPUT:

	VALUE OF PARAMETER		VALUE OF STANDARD DEVIATION	
	GUESSED	FITTED	ABSOLUTE	RELATIVE
K	1.0E08	3.0525E09	NOT FOUND	NOT FOUND
FL	1.0000	0.94708	NOT FOUND	NOT FOUND
AEXT	0.0100	0.01205	NOT FOUND	NOT FOUND

CURVE FITTING IN ORIGINAL COORDINATES:					IN NORMALIZED COORDINATES:		
		ABSORBANCE				MOLE	RATIO
I	METAL	MEASURED	CALCUL.	RESIDUAL	ALPHA	(M/L)	(L/M)
1	4.94E-6	0.6296	0.6296	0.0000	0.0615	0.0316	31.6332
2	1.45E-5	0.5510	0.5510	0.0000	0.1809	0.0926	10.7963
3	2.41E-5	0.4736	0.4724	0.0012	0.3003	0.1543	6.4822
4	3.37E-5	0.3986	0.3966	0.0020	0.4155	0.2159	4.6315
5	4.34E-5	0.3249	0.3248	0.0001	0.5246	0.2779	3.5990
6	4.82E-5	0.2929	0.2920	0.0009	0.5745	0.3065	3.2411
7	5.31E-5	0.2618	0.2614	0.0004	0.6210	0.3395	2.9454
8	5.55E-5	0.2477	0.2472	0.0004	0.6425	0.3550	2.8169
9	5.79E-5	0.2336	0.2342	−0.0006	0.6624	0.3702	2.7014
10	6.03E-5	0.2205	0.2215	−0.0010	0.6817	0.3860	2.5908
11	6.27E-5	0.2083	0.2102	−0.0018	0.6989	0.4012	2.4928
12	6.51E-5	0.2013	0.1995	0.0019	0.7151	0.4167	2.4001
13	6.75E-5	0.1881	0.1897	−0.0016	0.7300	0.4321	2.3141
14	6.99E-5	0.1801	0.1809	−0.0009	0.7433	0.4473	2.2356
15	7.23E-5	0.1699	0.1728	−0.0028	0.7557	0.4628	2.1607
16	7.47E-5	0.1649	0.1653	−0.0004	0.7670	0.4783	2.0908
17	7.72E-5	0.1568	0.1586	−0.0017	0.7773	0.4939	2.0252
18	8.20E-5	0.1468	0.1468	0.0000	0.7952	0.5245	1.9068
19	8.32E-5	0.1437	0.1437	0.0000	0.7998	0.5324	1.8785
20	8.56E-5	0.1439	0.1439	0.0000	0.7996	0.5478	1.8253
21	8.68E-5	0.1450	0.1371	0.0079	0.8100	0.5554	1.8004
22	9.16E-5	0.1265	0.1288	−0.0023	0.8225	0.5864	1.7053
23	9.64E-5	0.1226	0.1219	0.0008	0.8331	0.6171	1.6206
24	1.01E-4	0.1156	0.1159	−0.0003	0.8422	0.6481	1.5431

FITNESS TEST BY THE STATISTICAL ANALYSIS OF RESIDUALS:

RESIDUAL MEAN	= 8.799E-5
MEAN RESIDUAL	= 0.0012
STANDARD DEVIATION	= 0.0020
SKEWNESS	= 2.348
KURTOSIS	= 10.281
PEARSON'S CHI-SQUARE OBSERVED	= 8.00
THEORETICAL	= 12.60 (FOR 6 D.F. AND 0.95 PROBABILITY LEVEL)
HAMILTON'S R-FACTOR	= 0.007256

Table 7. Results calculated for Example 2 [$r = 10^3(A_{exp} - A_{calc})$]

Wavelength (in nm)					510		520		540	
A_0					1.210		1.200		0.550	
i	v_M(ml)	$c_M \times 10^5$	q_M	α	A_{exp}	r	A_{exp}	r	A_{exp}	r
1	0.0432	0.733	0.0727	0.0728	1.162	0.0	1.134	0.0	0.516	0.0
2	0.1731	2.934	0.2911	0.2888	1.019	1.5	0.937	2.4	0.414	0.4
3	0.2165	3.670	0.3641	0.3609	0.971	1.1	0.872	3.0	0.381	1.2
4	0.2595	4.399	0.4364	0.4322	0.924	0.9	0.806	1.6	0.347	0.4
5	0.3031	5.138	0.5098	0.5041	0.878	1.9	0.742	2.5	0.314	0.9
6	0.3464	5.871	0.5824	0.5749	0.831	1.1	0.677	1.2	0.281	0.8
7	0.3894	6.600	0.6548	0.6446	0.785	0.4	0.613	−0.3	0.248	0.2
8	0.4330	7.340	0.7281	0.7141	0.738	−1.5	0.549	−2.0	0.214	−1.5
9	0.4762	8.072	0.8008	0.7808	0.694	−2.2	0.488	−3.1	0.183	−1.2
10	0.5195	8.805	0.8735	0.8433	0.652	−3.3	0.430	−4.2	0.153	−1.6
11	0.5629	9.541	0.9465	0.8975	0.620	1.6	0.384	1.0	0.131	2.5
12	0.6059	10.270	1.0188	0.9364	0.592	2.2	0.347	4.1	0.110	0.8
13	0.6925	11.740	1.1356	0.9665	0.559	−1.1	0.301	−1.6	—	—
$\beta_{11} \times 10^{-6}$					1.651 ± 0.278		1.743 ± 0.314		3.043 ± 1.064	
f_L					0.938 ± 0.005		0.941 ± 0.005		0.911 ± 0.009	
A_{ext}					0.5576 ± 0.0050		0.2832 ± 0.0069		0.0932 ± 0.0073	
Residual mean					2.041E-4		3.553E-4		1.163E-4	
Mean residual					0.0014		0.0021		0.0009	
Standard deviation					0.0017		0.0024		0.0011	
Skewness					−0.354		0.075		0.536	
Kurtosis					2.001		1.924		2.617	
Pearson's chi-square observed					2.38		4.85		2.67	
theoretical					12.60		12.60		12.60	
Hamilton's R-factor					0.00199		0.00353		0.00385	

Table 8. Shortened MRLET output for Example 1 (Table 3)

MRLET PROGRAM

MRLET-TITLE: ZN + SNAZOXS, pH = 5.5, I = 0.1, 575 NM, 1.000 CM, HEURISTIC S.
TYPE OF INPUT: EXPERIMENTAL DATA

xxxxxx RURIK = 6 xxxxxx DATA
EXPERIMENTAL CONDITIONS:

METAL—TOTAL CONCENTRATION IN BURETTE (MOL/L):	0.002000
LIGAND—TOTAL CONCENTRATION IN VESSEL (MOL/L):	0.000165
INITIAL ABSORBANCE:	0.6700
INITIAL VOLUME (ML):	4.000
FACTOR OF MICROBURETTE (ML PER MM):	0.988
CURVE SEGMENT LIMITS—ALPHAMIN:	0.0100
—ALPHAMAX:	0.9900
TESTED STOICHIOMETRIC COEFFICIENTS P = ... Q = ...	1 2

POINTS OF EXPERIMENTAL A–V CURVE:

TITRATION DATA			SELECTED POINTS OF SEGMENT		
I	V(ML)	A READ	J	METAL(MOL/L)	A CORRECTED
1	0.0099	0.6280	1	0.00000494	0.6296
2	0.0289	0.5470	2	0.00001447	0.5510
3	0.0482	0.4680	3	0.00002411	0.4736
4	0.0675	0.3920	4	0.00003374	0.3986
5	0.0868	0.3180	5	0.00004342	0.3249
6	0.0964	0.2860	6	0.00004821	0.2929
7	0.1061	0.2550	7	0.00005306	0.2618
8	0.1110	0.2410	8	0.00005548	0.2477
9	0.1157	0.2270	9	0.00005785	0.2336
10	0.1206	0.2140	10	0.00006032	0.2205
11	0.1254	0.2020	11	0.00006269	0.2083
12	0.1302	0.1950	12	0.00006511	0.2013
13	0.1351	0.1820	13	0.00006753	0.1881
14	0.1398	0.1740	14	0.00006990	0.1801
15	0.1446	0.1640	15	0.00007232	0.1699
16	0.1495	0.1590	16	0.00007474	0.1649
17	0.1543	0.1510	17	0.00007716	0.1568
18	0.1639	0.1410	18	0.00008195	0.1468

Table 8 (contd.)

19	0.1664	0.1380	19	0.00008319	0.1437
20	0.1712	0.1380	20	0.00008561	0.1439
21	0.1736	0.1390	21	0.00008680	0.1450
22	0.1833	0.1210	22	0.00009164	0.1265
23	0.1929	0.1170	23	0.00009643	0.1226
24	0.2025	0.1100	24	0.00010127	0.1156

xxxxxx RURIK = 7 xxxxxx LASK (INITIAL GUESS):
NUMBER OF ESTIMATED PARAMETERS = 4
NUMBER OF POSITIVE PARAMETERS = 4
NUMBER OF TWIST MATRIX ELEMENTS IN DATA = 0
INITIAL GUESS OF THE FIRST PARAMETER (AEXT) = 0.01
INITIAL GUESS OF THE SECOND PARAMETER (FL) = 0.90
INITIAL GUESS OF THE THIRD PARAMETER (LOG K) = 9.00
INITIAL GUESS OF THE FOURTH PARAMETER (Q) = 2

xxxxxx RURIK = 3 xxxxxx STEG (STEPS OF PARAMETERS):
3 0.06

xxxxxx RURIK = 2 xxxxxx UTTAG (RESIDUAL-SQUARE SUM):
U = 4.14349E-02 PARAMETERS = 0.01000 0.90000 9.00000 2.00000

xxxxxx RURIK = 5 xxxxxx SKOTT (SHOT):
DET = 1.48072E-03
SIGY (STANDARD DEVIATION IN Y): 1.8379E-02
KBOM (PARAMETERS)

NUMBER	VALUE	DARR1	DARR2
3	9.28609E0	2.86574E-02	2.86574E-02

xxxxxx RURIK = 3 xxxxxx STEG (STEPS OF PARAMETERS):
1 0.04 2 0.01 3 0.2

xxxxxx RURIK = 2 xxxxxx UTTAG (RESIDUAL-SQUARE SUM):
U = 2.19640E-02 PARAMETERS = 0.01000 0.90000 9.28609 2.00000

xxxxxx RURIK = 5 xxxxxx SKOTT (SHOT):
DET = 1.41416E-09
SIGY (STANDARD DEVIATION IN Y): 2.9333E-03
KBOM (PARAMETERS)

NUMBER	VALUE	DARR1	DARR2
1	2.29424E-03	2.25108E-03	2.25108E-03
2	9.26916E-01	5.13708E-03	5.13708E-03
3	9.37156E 00	1.19959E-02	1.19959E-02

DET = 5.91280E-07
SIK (TWIST MATRIX ELEMENTS)
1 2 −1.74808E-01
1 3 1.55984E-01
2 3 −1.11772E-01

xxxxxx RURIK = 2 xxxxxx UTTAG (RESIDUAL-SQUARE SUM):
U = 3.37861E-04 PARAMETERS = 0.00249 0.92692 9.37156 2.00000

ALGORITHMIC PROCESS:

1 ITERATION	U = 3.37861E-04	PAR. = 0.00249	0.92692	9.37156	2.00000
2 ITERATION	U = 1.20580E-04	PAR. = 0.00110	0.94758	9.47373	2.00000
..	.....	.....	.....	.....	.....
17 ITERATION	U = 5.40662E-06	PAR. = 0.01337	0.94569	9.49520	2.00000

xxxxxx RURIK = 13 xxxxxx SKRIK (OUTPUT):
PARAMETERS AND THEIR STANDARD DEVIATIONS:

AEXT	= 0.01337	±0.00012
FL	= 0.94569	±0.00013
LOG K	= 9.49520	±0.00009
Q	= 2.00000	± −1.00000

CURVE FITTING IN ORIGINAL AND NORMALIZED COORDINATES:

I	V(EXP)	A(EXP)	A(ACAL)	RESIDUAL	METAL	M/L	ALPHA
1	0.00988	0.6296	0.6296	0.0000	4.94E-6	0.0317	0.0616
2	0.02895	0.5510	0.5510	0.0000	1.45E-5	0.0928	0.1813
3	0.04821	0.4736	0.4724	0.0012	2.41E-5	0.1545	0.2990
4	0.06748	0.3986	0.3966	0.0020	3.37E-5	0.2162	0.4133
5	0.08685	0.3249	0.3247	0.0002	4.34E-5	0.2783	0.5256
6	0.09643	0.2929	0.2918	0.0011	4.82E-5	0.3090	0.5743
7	0.10611	0.2618	0.2612	0.0006	5.31E-5	0.3400	0.6217
8	0.11095	0.2477	0.2470	0.0007	5.55E-5	0.3555	0.6432
9	0.11569	0.2336	0.2339	−0.0003	5.79E-5	0.3707	0.6647
10	0.12063	0.2205	0.2212	−0.0007	6.03E-5	0.3866	0.6846

Contd.

Table 8 (contd.)

11	0.12538	0.2083	0.2099	−0.0015	6.27E-5	0.4017	0.7031
12	0.13022	0.2013	0.1992	0.0021	6.51E-5	0.4173	0.7137
13	0.13506	0.1881	0.1894	−0.0013	6.75E-5	0.4328	0.7338
14	0.13980	0.1801	0.1807	−0.0006	6.99E-5	0.4480	0.7461
15	0.14464	0.1699	0.1725	−0.0026	7.23E-5	0.4635	0.7616
16	0.14948	0.1649	0.1651	−0.0002	7.47E-5	0.4790	0.7692
17	0.15433	0.1568	0.1584	−0.0015	7.72E-5	0.4945	0.7815
18	0.16391	0.1468	0.1468	0.0000	8.20E-5	0.5252	0.7968
19	0.16638	0.1437	0.1437	0.0000	8.32E-5	0.5331	0.8015
20	0.17122	0.1439	0.1392	0.0047	8.56E-5	0.5486	0.8012
21	0.17359	0.1450	0.1371	0.0079	8.68E-5	0.5562	0.7995
22	0.18327	0.1265	0.1288	−0.0022	9.16E-5	0.5873	0.8276
23	0.19286	0.1226	0.1219	0.0007	9.64E-5	0.6180	0.8336
24	0.20254	0.1156	0.1159	−0.0003	1.01E-4	0.6490	0.8444

FITNESS TEST BY THE STATISTICAL ANALYSIS OF RESIDUALS:

RESIDUAL MEAN	= 8.241E-05
MEAN RESIDUAL	= 0.0010
STANDARD DEVIATION	= 0.0015
SKEWNESS	= 1.063
KURTOSIS	= 5.002
PEARSON'S CHI-SQUARE OBSERVED	= 4.00
THEORETICAL	= 12.60 (FOR 6 D.F. AND 0.95 PROBABILITY LEVEL)
HAMILTON'S R-FACTOR	= 0.005379

Systematic variation of m particular parameters is done in block LETA, called by RURIK = 5. The label SKOTT introduces the "shots" fired for individual parameters in a particular step, and the relevant values of U are printed in tabular form. In each iteration $(m+1)\,(m+2)/2$ shots are performed, and after each iteration, current estimates of the parameters and statistics are printed: SIGY means the value of the standard deviation of the dependent variable $s(A)$ in the given iteration, KBOM lists the refined values of the parameters, and DARR2 their standard deviations.

The twist matrix is interpreted as a rotation of the axis to coincide with the long axis of the ellipse. When parameters are varied along the direction of the main axes of the ellipsoidal cross-section of the pit, the convergence will be improved. For this purpose the axes of the trial parameters must be transformed, and the elements above the diagonal of a new (twist) matrix are printed after the label SIK. The PROVA block performs a test of the U value reached in a particular iteration. The lowest value of U, once found, is stored. If the calculated set of trial parameters in the ith iteration gives a lower value of the U function than any previously found, it is accepted as the best set; if, however, the preceding $(i-1)$th iteration gave a lower value of U, this previous set is accepted and printed by the label GAMLA KONSTANTER. If some earlier iteration gave the lowest U value, then *that* set of parameters is accepted and printed by the label SLUMPSKOTT.

The most efficient minimization strategy seems to be a combination of a heuristic and an algorithmic process. The ill-conditioned parameters, for example, are heuristically refined at the beginning of the minimization, and later an algorithmic refinement of all the parameters is performed.

When one of the termination criteria is fulfilled, the minimization process terminates. Label SKRIK produces a table of final estimates of the refined parameters, with their standard deviations and the degree-of-fit table produced by the statistical analysis of residuals. These tables are the same as in MRFIT.

The purpose of studying simulated data is (1) investigation of the influence of the instrumental error of the spectrophotometer used on the estimates of parameters refined, or (2) investigation of ill-conditioned parameters, or (3) testing the program validity and the reliability of parameter estimation. In the simulated data, the random error generated is used to load precise absorbance values. The resulting spread of points along the A *vs.* q_M curve is a good representation of real experimental data.

Table 9 shows how the calculated estimates of the three parameters depend on the simulated instrumental error, $s_{inst}(A)$. The set of generated random errors is statistically tested in order to find whether its distribution is Gaussian. The reliability of the calculated parameter estimates may be classified according to the agreement between the statistical characteristics of the set of random errors and the set of residuals. The original mole-ratio curve, along which the random errors are spread, should be identical with the calculated mole-ratio curve, so each residual should be of the same magnitude as the corresponding random error of the particular point but of opposite sign.

Because all the computational conditions were kept constant, and only $s_{inst}(A)$ was changed. Table 9 illustrates the effect of randomization. Well-conditioned parameters are estimated accurately within the tolerance of their standard deviations but ill-conditioned parameters are estimated with a great deal of uncertainty and not accurately. This illus-

Table 9. Influence of the instrumental error of the spectrophotometer on the values found for the parameters (Example 3)

$s_{inst}(A)$	1.0*E*-6	0.0001	0.001	0.002	0.004	0.006
			STATISTICAL ANALYSIS OF GENERATED ERRORS			
DEVIATION MEAN	4.26E-9	3.45E-6	3.47E-5	6.94E-5	1.39E-4	2.08E-4
MEAN DEVIATION	6.00E-7	6.50E-5	0.00065	0.00129	0.00258	0.00387
STAND. DEVIATION	8.00E-7	7.80E-5	0.00078	0.00156	0.00312	0.00468
SKEWNESS	0.445	0.550	0.551	0.551	0.551	0.551
KURTOSIS	3.06	3.05	3.05	3.05	3.05	3.05
CHI-SQUARE (to be 12.6)	1.71	4.57	4.57	4.57	4.57	4.57
			RESIDUAL-SQUARE SUM AT ACHIEVED PIT			
U	3.20E-10	6.43E-8	6.34E-7	2.53E-6	1.01E-5	2.37E-5
s(A)	3.58E-6	5.07E-5	0.00016	0.00032	0.00064	0.00097
			VALUES OF ESTIMATED PARAMETERS			
$\beta_{11} \times 10^{-6}$	0.997 ± 0.000	0.999 ± 0.004	1.021 ± 0.044	1.045 ± 0.089	1.096 ± 0.185	1.218 ± 0.306
f_L	1.000 ± 0.000	1.000 ± 0.000	0.996 ± 0.006	0.996 ± 0.006	0.993 ± 0.012	0.988 ± 0.017
A_{ext}	1.000 ± 0.000	1.000 ± 0.000	0.992 ± 0.014	0.992 ± 0.014	0.983 ± 0.028	0.966 ± 0.039
			STATISTICAL ANALYSIS OF RESIDUALS			
RESIDUAL MEAN	7.11E-7	2.85E-6	2.87E-6	3.83E-6	8.10E-6	−5.94E-5
MEAN RESIDUAL	3.73E-6	5.96E-5	0.000588	0.00117	0.00235	0.00367
STAND. DEVIATION	5.34E-6	7.58E-5	0.000752	0.00150	0.00300	0.00459
SKEWNESS	1.384	0.283	0.178	0.173	0.173	0.122
KURTOSIS	6.14	3.01	3.09	3.09	3.09	2.91
CHI-SQUARE (to be 12.6)	8.57	4.57	3.43	3.43	2.86	5.71
R-FACTOR	0.000009	0.00013	0.00129	0.00258	0.00516	0.00789

Table 10

	LETAGROP-SPEFO[14]	MRLET	MRFIT
(1) Mathematical model	Mass-balance equations and Beer-Lambert Law	Recursive expression for photometric titration curve	
(2) Parameters refined			
Stability constants β_{pq} of:	All consecutive complexes	One prevailing complex ML_q	
Molar absorptivities ε_{pq} of:	All consecutive complexes	One prevailing complex ML_q	
Stoichiometric coefficients p, q:	For each complex by means of SPECIES SELECTOR[14]	$p = 1$, q to be estimated	p and q are known constants
Effective concentration factor of ligand used, f_L	No	Yes	Yes
(3) Minimization procedure	LETAGROP program[14]	LETAG routine[10]	FIT routine[13]
(4) Strategy of minimization process	Heuristic only	Heuristic and/or algorithmic	Algorithmic only
(5) Estimation reliability of parameters			
Statistical tests	No	Yes	Yes
(6) Simulation of experimental data	No	Yes	Yes
(7) Plot of fitted mole-ratio graph	No	Yes	Yes

trates the danger of applying algorithmic non-linear regression to experimental data, when the true values of the parameters sought are unknown.

CONCLUSIONS

The programs MRLET and MRFIT offer considerable advantages over LETAGROP-SPEFO,[14] the most obviously comparable program. Table 10 compares and contrasts some of the features of the three programs.

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