

# Chemical model building and testing: Spectrophotometry

**S**PECTROPHOTOMETRY and potentiometry are two useful methods for elucidating the composition of solutions. The power of the techniques lies in the fact that quantitative absorbance or pH measurement can be performed without disturbing the complexation equilibria under consideration. Equilibria studies of complexes involve finding a chemical model of the complex-forming system and determining the number of complexes in solution, their stoichiometry, stability constants, and molar absorptivities.

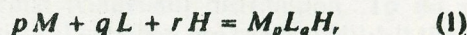
The spectrophotometric and potentiometric study of solution equilibria has been greatly advanced by the introduction of computer-assisted methods of data processing.<sup>1-10</sup> The computer is connected to the primary instrument (spectrophotometer or pH meter) with a predetermined function, the computer function being limited by the intelligence of a program and by the power of the logic tools used in a program. Thus, the computer is both more versatile and more difficult to use.

Computer-assisted analysis of solution equilibria has made great progress during the last 25 years; we now have more appropriate numerical methods available than those developed by Ingri and Sillén.<sup>1</sup> To make any progress in equilibria study today, we must pay attention to the advanced numerical algorithms and structure of sophisticated program-sys-

tems. Computer interpretation of spectra thus enables a much better understanding of solution equilibria.

## Design of chemical model

Considering a system of metal  $M$ , ligand  $L$ , and proton  $H$  in which  $M$  and  $L$  absorb radiation in the visible and/or ultraviolet (UV) region, chemical interaction can lead to complexes represented by:



with the overall stability constant

$$\beta_{pqr} = [M_pL_qH_r]/([M]^p[L]^q[H]^r) \quad (2)$$

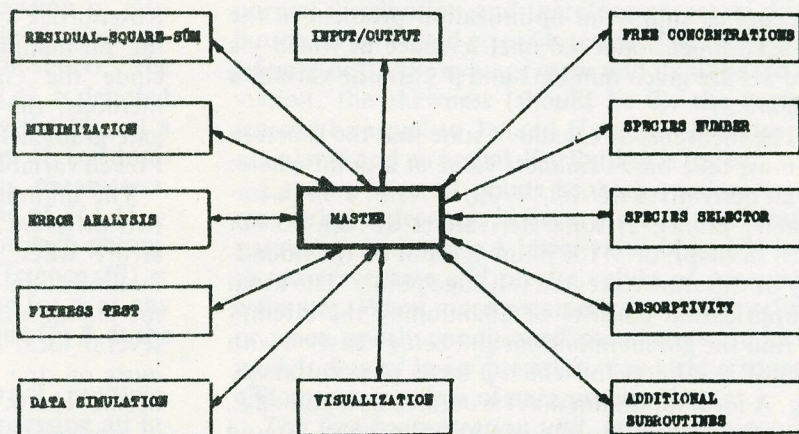
From Lambert-Beer's law it is obvious that for the  $i^{\text{th}}$  solution at the  $k^{\text{th}}$  wavelength, the absorbance,  $A_{i,k}$ , for unit pathlength is defined by:

$$A_{i,k} = \sum_0^P \sum_0^Q \sum_0^R [M_pL_qH_r] \cdot \epsilon_{pqr,k} \\ = \sum_0^P \sum_0^Q \sum_0^R \beta_{pqr} [M]^p [L]^q [H]^r \cdot \epsilon_{pqr,k} \quad (3)$$

where  $PQR$  are the maximum value of possible stoichiometric indices that they can reach, and  $\epsilon_{pqr,k}$  is the molar absorptivity at  $k^{\text{th}}$  wavelength for the complex species  $M_pL_qH_r$ . Thus, given sufficient values of  $A_{i,k}$  it should be possible to evaluate  $\beta_{pqr}$  and  $\epsilon_{pqr}$  for the designed chemical model.

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Figure 1 The structure of a general program for chemical model building and testing by regression analysis of spectrophotometric data. (Reproduced with permission from Talanta 33, MS862 [June 1986].)





### Programs for chemical model search

The advanced computer program of spectral analysis is constructed from twelve logical units, each having a specific function and each consisting of several subroutines. This structural classification (Figure 1) of a sophisticated program seems to be valuable in understanding the anatomy and modus operandi of a program and its further implementation.<sup>1-4,8-10</sup>

**The residual-square-sum unit.** The spectrophotometric equilibrium program adjusts parameters  $\beta_{pqr}$  and  $\epsilon_{pqr}$  for any absorbance data by minimizing the residual-square-sum function  $U$  defined by

$$\begin{aligned}
 U &= \sum_{i=1}^{n_s} \sum_{k=1}^{n_w} (A_{\text{exp},i,k} - A_{\text{calc},i,k})^2 \\
 &= \sum_{i=1}^{n_s} \sum_{k=1}^{n_w} (A_{\text{exp},i,k} - \sum_{j=1}^{n_c} \epsilon_{k,j} c_j)^2 \\
 &= \text{minimum}
 \end{aligned} \quad (4)$$

where the dependent variable  $A_{i,k}$  represents the element of the absorbance matrix of size  $(n_s \times n_w)$  and the independent variables are adjustable total concentrations of basic components  $c_M$ ,  $c_L$ , and  $c_H$  being adjusted in  $n_s$  solutions during the experiment. Unknown parameters might be divided into three groups: 1) the number of light-absorbing complexes  $n_c$ , and their stoichiometry  $(p, q, r)$ ,  $j = 1, n_c$ ; 2) stability constants and molar absorptivities of all complexes  $\beta_{pqr,j}$  ( $\epsilon_{pqr,j,k}$ ,  $k = 1, n_w$ ),  $j = 1, n_c$ ; 3) free concentrations of all complexes  $[M_p L_q H_r]$ ,  $j = 1, n_c$ , in all  $n_s$  solutions.

**The minimization unit.** The regression problem is to find the best estimates of parameters,  $\beta_j$ ,  $j = 1, m$ , for which a residual-square-sum function  $U = \sum (y_{\text{exp},i} - y_{\text{calc},i})^2$  takes on its smallest value where  $y_{\text{calc}} = f(x; \beta_1, \beta_2, \dots, \beta_m)$ . The nonlinear estimation appears as simply an optimization problem in the  $(m+1)$ -dimensional parametric space in which  $y$ 's and  $x$ 's are given numbers and  $\beta$ 's are the variables (Figure 2).

The theorems of calculus<sup>11</sup> state that the function  $U$  must take on its smallest value at a point where: 1) all derivatives  $dU/d\beta_j = 0$ ,  $j = 1, \dots, m$  (a stationary point); 2) some derivatives  $dU/d\beta_j$  do not exist (a cusp); or 3) the point  $\beta_j$  is not on the boundary of the allowed region (an edge point). The usual simplification consists of abandoning the attempt to find the global minimum and being satisfied with a local minimum for which  $\beta$  has a physical meaning. A local minimum may be defined as a point  $\beta_{\text{min}}$  where for all points in some neighborhood around  $\beta_{\text{min}}$  we have  $U(\beta) > U(\beta_{\text{min}})$ .

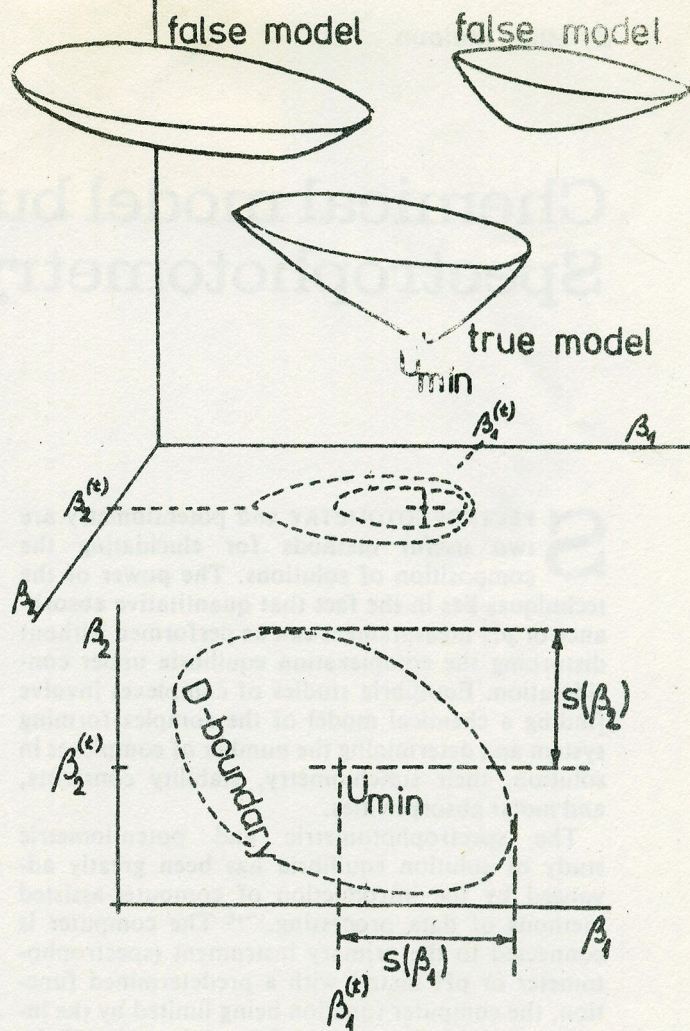


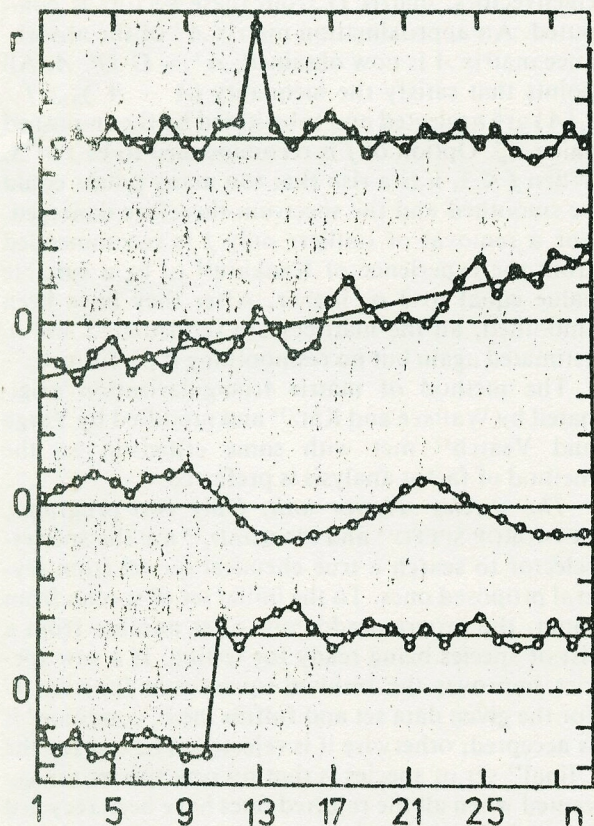
Figure 2 Graphical interpretation of a systematic search of a minimum  $U_{\text{min}}$  in  $(m+1)$ -dimensional parametric space, for two parameters  $m = 2$ , i.e., 3-D space. Parametric standard deviations  $s(\beta_1)$  and  $s(\beta_2)$  are estimated from the last  $U$  contour "D boundary."

There are more than 40 various optimization algorithms,<sup>11-13</sup> all of which fall into two broad classes: derivative-free methods and derivative methods. If in the search for a minimum of  $U$ , the partial derivatives of  $U$  with respect to  $\beta_j$  must be calculated, the method is a derivative method; otherwise, the method is termed derivative-free.

Some derivative-free methods are: direct searching (Grid search method, Monte Carlo method, Rosenbrock's method); the simplex method; and the pit-mapping method. Derivative methods include the Gauss-Newton and Newton-Raphson methods, the steepest descent method, the conjugate gradients method, and the Davidson-Fletcher-Powell variable metric method.

The minimization process may be organized by two different algorithmic and heuristic (trial-and-error) ways. The algorithmic process performs minimization quite automatically and usually finds the global minimum or the lowest minimum from several local ones. The heuristic process depends more on decisions of the user. The user must then decide whether it is sufficient to know the location of any local minimum or whether the knowledge of the global minimum is required. The user should





**Figure 3** Graphical interpretation of residuals quickly shows (from top to bottom): 1) an extreme observation (an outlier); 2) a trend in the residuals; 3) the frequency of sign changes; and 4) an abrupt shift level of the experiment.

guess the initial parameters, minimization steps, and organizational framework to control the process from iteration to iteration.<sup>7-9,11-13</sup>

**The error analysis unit.** There are several statistical measures corresponding to a precision of estimated parameters and a confidence interval of calculated regression curve. In LETAGROP-SPEFO,<sup>14</sup> the standard deviation for absorbance,  $s(A)$ , is denoted as SIGY and is calculated by dividing the  $U_{\min}$  at a minimum by the number of degrees of freedom. For standard deviations of various parameters,  $s(\beta)$ , the "D boundary" last contour of  $U$ ,<sup>7</sup>  $U = U_{\min} + s^2(A)$ , is defined and  $s(\beta_i)$  for each parameter is calculated as the maximum difference  $s(\beta) = \max [(\beta_D - \beta_{\min})_i]$  between the value for  $\beta_i$  at any point on the D boundary and the value for  $\beta_i$  at the minimum (Figure 2).

PSEQUAD<sup>15</sup> also evaluates the partial, multiple, and total correlation coefficients expressing an interdependence of two parameters  $\beta_i$  and  $\beta_j$ . A cor-

relation coefficient of zero signals the complete independence of the species while +1 or -1 means a complete correlation between them, and two such parameters cannot be estimated simultaneously.

**The fitness test unit.** To identify the "best" chemical model when more than one is possible or proposed, the analysis of residuals,  $r_i = y_{\text{exp},i} - y_{\text{calc},i}$ , is performed. If the model represents the data adequately, the residuals should be randomly distributed about  $y_{\text{calc}}$  predicted by the regression equation. While graphical presentation of residuals (Figure 3) will quickly discover an extreme observation (an outlier), a trend in the residuals, the sign changes, and an abrupt shift level of the experiment, the statistical analysis makes a rigorous test of achieved degree-of-fit if residuals are represented by a normal (Gaussian) distribution. To do so, some statistical variables are calculated:<sup>16</sup> the residual mean, the mean residual and its standard deviation, the skewness (which should be zero), the kurtosis (which should be equal to 3), and the Pearson Chi<sup>2</sup> test. The Hamilton R-factor is a measure of relative fitness expressed as a percentage. A value less than 0.5% describes an excellent fitness achieved while greater than 2% expresses a poor fit.

**The data simulation unit.** To test the suitability of a program function for a particular equilibrium case, simulated data are often used. For optional values of parameters (i.e., stability constants, molar absorptivities, etc.), and for given values of independent variables (i.e., concentrations, pH, etc.), the "theoretical" points of the curve are precisely calculated. Each "theoretical" point is then transformed into an "experimental" one by the addition of a random error,  $e$ , generated by the random numbers generator and added to the basic instrumental error of the spectrophotometer used,  $s_{\text{inst}}(A)$ , given in input data.

Generated random errors should have a Gaussian normal distribution and therefore an actual distribution of generated errors is tested. The mean error (should be 0), the average error and its standard deviation, the skewness (should be 0), the kurtosis (should be equal to 3), and Hamilton R-factor are calculated and an actual distribution is tested.

Loading spectral points by high random errors may bring a decrease in accuracy and precision of parametric estimates. A lower value of  $s_{\text{inst}}(A)$  leads to more accurate and precise values of parametric estimates. When more parameters are to be refined or when poorly conditioned parameters are in the model, data of lower precision may yield erroneous and uncertain values of parametric estimates.

**The free concentration unit.** In solution equilibria studies the calculation of free concentration of



each species in a complex-forming system is based on the total (analytical) concentrations of basic components and the stability constants. The chemical model is defined by stoichiometric coefficients ( $p, q, r$ ),  $j = 1, n_c$ . Species concentrations expressed in mass-balance equations are calculated for each experimental absorbance-concentration curve point and various approaches may be found.<sup>17,18</sup> Some are based on COGS subroutine<sup>17</sup> when free metal, ligand, and proton concentrations are calculated by the derivative-free, iterative method: Starting from an initial guess that complexation is negligible,  $[M] = c_{M,exp}$ ,  $[L] = c_{L,exp}$ , and  $[H] = c_{H,exp}$ , the method calculates concentration for each complex to give  $c_{M,calc}$ ,  $c_{L,calc}$ , and  $c_{H,calc}$ . The initial estimates of  $[M]$ ,  $[L]$ , and  $[H]$  are then replaced by  $[M] \cdot (c_{M,exp}/c_{M,calc})^{1/2}$ ,  $[L] \cdot (c_{L,exp}/c_{L,calc})^{1/2}$ , and  $[H] \cdot (c_{H,exp}/c_{H,calc})^{1/2}$ . With these new estimates the calculations are repeated until all values of calculated total concentrations differ from experimental ones by less than a specified quantity (usually 0.001% of  $c_{M,exp}$ , etc.).

**The species number unit.** There is a theorem in the matrix calculus that the rank of matrix which can be written as the product of two other matrices is equal to the smaller of the ranks of two latter matrices. As the rank of the absorbance matrix was proven to be equal to or less than a number of absorbing species in solutions,<sup>19-21</sup>  $n_c' \leq n_c' \leq n_c + n_l$ , it is useful to determine the integer  $n_c'$  at the beginning of spectral analysis.

The second moment matrix is formed from the product of absorbance matrix  $A$  and its transpose  $A^T$  by the formula  $M = (1/n_c) \cdot A \cdot A^T$ , which leads to a symmetric square matrix of order  $n_c$  and of rank  $n_c'$ , rank  $(M) \leq n_c'$ . Wernimont<sup>20</sup> related that the matrix  $M$  has at most  $n_c'$  nonzero eigenvalues, and the number of nonzero eigenvectors is the mathematical rank of the matrix  $M$ .

As each element of the absorbance matrix  $A$  is subject to experimental error, the number of nonzero eigenvalues is  $\min(n_w, n_c')$ . Let the eigenvalue of  $M$  be  $\alpha_i$  and let the independent components in the system be  $k$ . The residual standard deviation of absorbance is given by:

$$s_k(A) = [(tr(M) - \sum_{i=1}^k \alpha_i) / (n_w - k)]^{1/2} \quad (5)$$

When the precision of the absorbance measurement  $s_{inst}(A)$  is determined by the instrumental error of the spectrophotometer used, it may be set so that if  $s_k(A) \leq s_{inst}(A)$ , then  $n_c' \leq k$ ;  $tr(M)$  is trace of matrix  $M$ , i.e., the sum of elements on diagonal of matrix.

Factor analysis FA608<sup>21</sup> can also be applied to find fatal, accidental errors in the absorbance ma-

trix. From second moment matrix  $M$  and its  $n_c'$  eigenvectors, matrix  $G$  from these vectors is computed. An approximation matrix  $A'$  of the absorbance matrix  $A$  is now obtained,  $A' = G \cdot G^T \cdot A$ . All points that satisfy the inequality  $(A - A')_{ij} > f \cdot s_k(A)$  are neglected and substituted by the smoothed value  $A'_{ij}$ . Option of  $f$  is recommended to be  $f = 3$ . When  $f < 3$ , it is a risk that too many points could be smoothed and the spectrum therefore damaged. For a removal of outliers only  $f$  is recommended from the experience of Kankare<sup>22</sup> to be a suitable value equal to 3 or higher. After they have been smoothed, all the matrices are recalculated and  $n_c'$  estimated again but no resmoothing is performed.

The method of matrix triangularization originated by Wallace and Katz<sup>21</sup> and modified by Varga and Veatch<sup>22</sup> met with some criticism, so the method of factor analysis is preferred.

**The species selector unit.** Only two programs, LETAGROP-SPEFO<sup>14</sup> and PSEQUAD,<sup>15</sup> use the species-selector to search a true chemical model from several proposed ones. To the initial set of species from input, the program adds one after another from a list of species being ready for testing. If a new species improves the residual-square-sum function  $U$  for the given data set and fulfills the  $F_0$  condition<sup>7</sup> it is accepted; otherwise it is rejected. The test for the "final" set of species is that no new species are accepted when all the rejected ones have been recycled through the program.

Sometimes the best estimates of  $\beta_{pqr}$  may come out quite low as compared with its standard deviation,  $s(\beta_{pqr})$ , and one may ask whether the value  $\log \beta_{pqr}$  is significant or not. Sillén<sup>9</sup> counts as "significant" only if  $\beta_{pqr} > F_0 \cdot s(\beta_{pqr})$ ; hence species which do not fulfill this condition are rejected. The  $F_0$  may be correlated to a value for the "level of confidence," which would mean the probability that the corresponding parameter would be significantly positive. The fact that a certain species has been rejected by the species selector does not mean that it does not exist, though the data analyzed give no strong evidence for its existence.

The unknown stoichiometry of complexes may be determined by the recently developed ESI method.<sup>19,24</sup> A group of complexes is divided into "certain" complexes of known stoichiometry and "uncertain" complexes; the stoichiometry and stability constants are estimated simultaneously and proven by achieved degree of fit. The real values found for stoichiometric indices should not differ significantly from integers if the chemical model found is right.

**The absorptivity unit.** The adjustment of stability constant  $\beta_{pqr}$  and molar absorptivities  $\epsilon_{pqr}$  in LETAGROP-SPEFO<sup>14</sup> is made at two levels. The  $\beta_{pqr}$

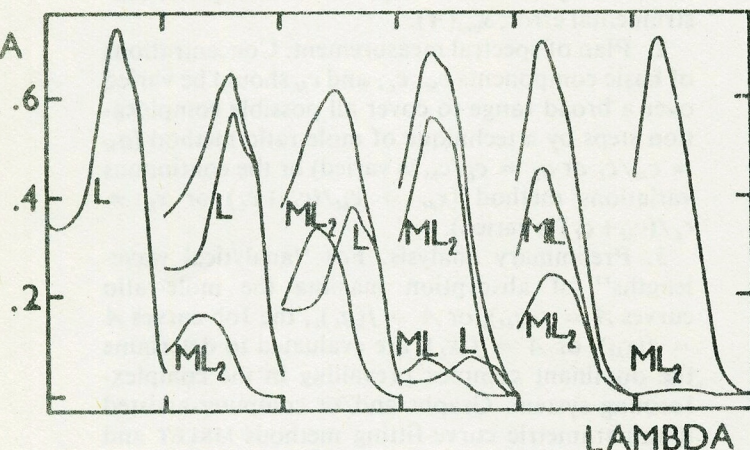


are varied at an upper level, and free concentrations of complex-species in each solution are calculated. Then, for each wavelength, the contribution to  $U$  for systematically varied values of  $\epsilon_{pqr}$  is calculated, assuming that the estimated  $\epsilon_{pqr}$  gives the minimum contribution to second-degree-surface function  $U$ . After each iteration for each wavelength, negative "insignificant" values of  $\epsilon_{pqr}$  are eliminated. SQUAD<sup>22</sup> employs the ECOEF subroutine, which calculates molar absorptivities  $\epsilon_{j,k}$  of the  $j^{\text{th}}$  species for the  $k^{\text{th}}$  wavelength with the use of an algorithm from Nagano and Metzler.<sup>24</sup>

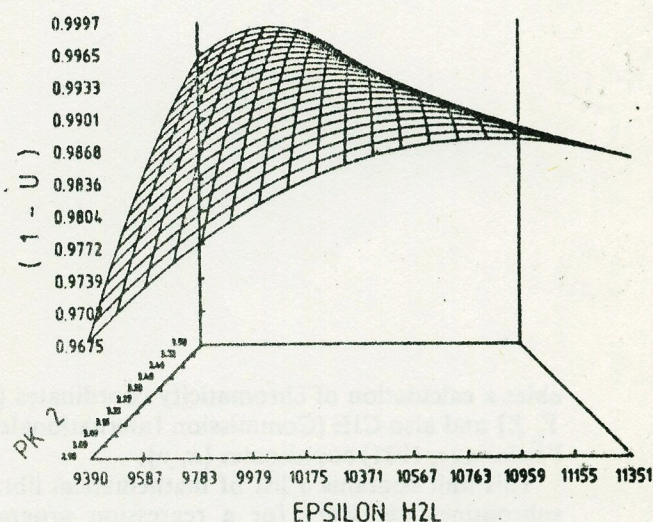
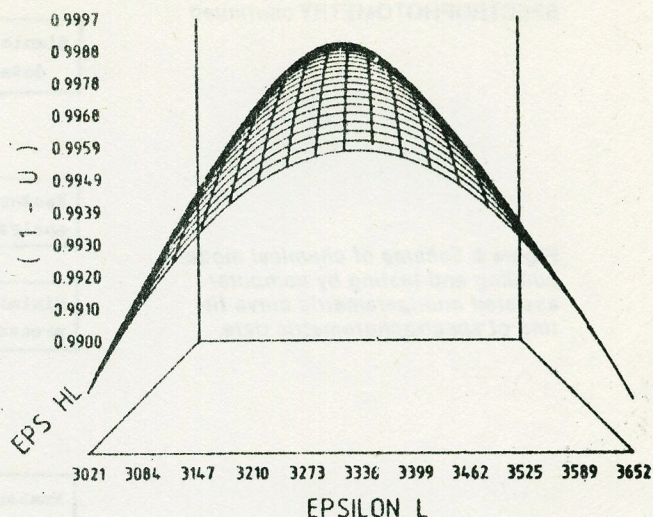
**The visualization tools unit.** A 3-D graph of an absorbance-response-plane visualizes absorbance changes within a variation of concentrations of basic components, helps to design an experimental plan of solution preparation, and also demonstrates a quality of fitness of calculated regression spectra through experimental points.

Printer-plot in SQUAD visualizes a degree-of-fit of each spectrum and its particular decomposition to the absorbance increments of individual species in the system. It is beneficial in experimental design to exclude those solutions with concentrations that indicate poor absorbance for some species (Figure 4).

The response-surface-plane of the residual-square-sum function  $U$  represents the paraboloid in two optional parametric coordinates in the neighborhood of the minimum (pit),  $U_{\min}$ . For two optional parameters, the shape of paraboloid ( $1.0 - U$ ) is investigated for conditioning parameters in the model. The regular paraboloid shape proves that both parameters are well-conditioned in a model and so their determination may lead to accurate



**Figure 4** Each experimental spectrum is decomposed into spectra of individual species of chemical model indicated by spectra in the complex-forming system. Spectra of six solutions are described by a simple system of  $ML_1$ ,  $ML$ , and  $L$  color complexes.



**Figure 5** Response-surface of residual-square-sum function ( $1 - U$ ) for two well-conditioned parameters in model (a), and for two ill-conditioned parameters in model (b) whose estimation is rather uncertain. Example of a model of two overlapping protonation equilibria by absorbance-pH curve analysis. (Reproduced with permission from *Talanta* 32, 973-986 [1985].)

and precise estimates. The "flat-bottomed-saucer" shape of the pit-neighborhood illustrates two ill-conditioned parameters; their determination is rather uncertain and regression analysis can lead to uncertain estimates only (Figure 5).

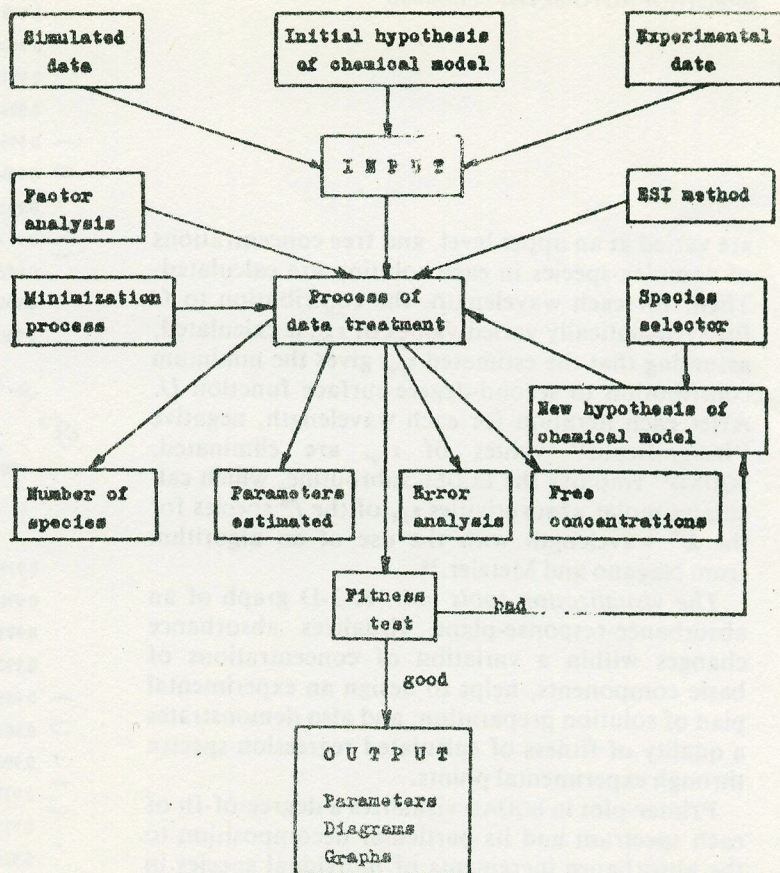
The  $U$  function contours may be plotted in the space of any two optional parameters at one time. This gives the detailed description of the shape of the  $U_{\min}$  neighborhood.

Physical meaning of estimated molar absorptivities is considered from a spectrum  $\epsilon = f(\lambda)$  for each light-absorbing species. Realistic and nonnegative values of  $\epsilon$  and the smooth shape of the spectrum are examined.

**The additional subroutine unit.** Reilley and Smith<sup>27</sup> extended complementary tristimulus colorimetry to a determination of equilibrium constants and of a formula of metal complexes for simple and consecutive reactions during which colored substances are present or are formed. SQUAD(84)<sup>28</sup> en-



**Figure 6** Schema of chemical model building and testing by computer-assisted multiparametric curve fitting of spectrophotometric data.



ables a calculation of chromaticity coordinates  $\{X, Y, Z\}$  and also CIE (Commission Internationale de l'Éclairage, 1931) coordinates  $\{x, y\}$ .

This unit contains a list of mathematical library subroutines necessary for a regression program. Format-free input reading routines are here.

**The input/output unit.** Some programs, such as SQUAD<sup>22</sup> and SQUAD(84),<sup>10</sup> use self-explanatory messages whenever fatal errors or other misprints on input cards are detected and program execution is interrupted. Even when spectra treated by computer analysis are from a spectrophotometer giving a high degree of precision, the selection of a certain range of data may be necessary. Often some species are present in low concentration. There may be "enough to interfere, but not enough to determine" a specimen. Unfortunately, there is no unified method of input preparation for handling such situations when more programs for a comparison are executed.

Numerical values of stability constants and molar absorptivities may not seem very interesting in output. The graphical interpretation brings greater effect as a diagnostic tool for the search of a most probable chemical model. Spectra-fitting, 3-D graph, graph of molar absorptivities and their standard deviations, graph of deconvolution of each experimental spectrum, distribution diagram of the relative population of all species, and graph of a 3-D response plane  $(1 - U)$  dependent on two optional parameters are the most frequently used graphs. The plane  $(1 - U)$  is a function of two chosen parameters.

### Procedure of chemical model building

The procedure of chemical model building and testing contains the experimental part followed by a computer-assisted analysis in a sequence (Figure 6):

1. Evaluation of instrumental error of the spectrophotometer,  $s_{\text{inst}}(A)$ . Wernimont's procedure<sup>20</sup> of spectrophotometer performance examination is applied on spectra measured for various concentrations of potassium dichromate in order to evaluate  $s_{\text{inst}}(A)$ . As the matrix rank for  $K_2Cr_2O_7$  is equal to one, the standard deviation  $s_k(A)$  estimated by factor analysis FA608<sup>23</sup> represents the requested instrumental error,  $s_{\text{inst}}(A)$ .

2. Plan of spectral measurement. Concentrations of basic components  $c_M$ ,  $c_L$ , and  $c_H$  should be varied over a broad range to cover all possible complexation steps by a technique of mole-ratio method ( $q_M = c_M/c_L$  or  $q_L = c_L/c_M$  is varied) or the continuous variations method ( $x_M = c_M/(c_M + c_L)$  or  $x_L = c_L/(c_M + c_L)$  is varied).

3. Preliminary analysis. For "analytical wavelengths" of absorption maxima the mole-ratio curves  $A = f(q_M)_h$  or  $A = f(q_L)_h$ , the Job curves  $A = f(x_M)_h$  or  $A = f(x_L)_h$  are evaluated to determine the dominant complex prevailing in the complex-forming system. Graphs and/or computer-assisted multiparametric curve-fitting methods MRLET and MRFIT,<sup>24</sup> JOBCON,<sup>25</sup> DCLET,<sup>26</sup> SPOPT,<sup>9</sup> LETAGROP-SPEFO,<sup>14</sup> NCLET,<sup>27</sup> and PSEQUAD<sup>15</sup> may be applied.

4. Number of species by factor analysis. Factor analysis FA608<sup>23</sup> estimates the number of light-absorbing species being equal to the rank of absorb-



ance matrix. Fatal, outliers points in the spectra are also detected by FA608, corrected, and rank determination repeated. Outliers-free spectra are then ready for chemical model determination.

5. Setting up chemical model hypothesis. To the dominant species denoted as "certain" complexes found by preliminary analysis, one new species after another from a list of suggested species is added and the resulting model tested. Stoichiometry of some "uncertain" complexes may also be determined by the ESI method.<sup>24</sup>

6. Diagnostic tools testing chemical model. After termination of minimization, some diagnostic tools are examined to accept or reject a tested hypothesis of a chemical model. Quite incorrect hypotheses may lead to divergency, cyclization, or failing of the minimization process. To set up a new hypothesis, the following diagnostics are considered: 1) physical meaning of estimates of parameters found or realistic values; 2) physical meaning of species concentrations calculated or realistic molarity in a

range from  $10^{-8}$  up to 10; 3) physical meaning of estimated standard deviations of parameters or realistic values; 4) parametric correlation coefficients express an interdependence of two parameters; 5) achieved degree-of-fit is a criterion of reliability of parametric estimates; 6) found real-values of stoichiometric indices by the ESI method<sup>24</sup> should be near integer values; 7) deconvolution of each experimental spectrum to absorbance increments of individual species helps to design a new experiment; and 8) 3-D graph of  $(1 - U)$  response surface, to examine the conditioning of the parameters in the model.

7. Search of best computation strategy. Analysis of simulated spectra enables: 1) a search of the best computational strategy of efficient regression analysis; 2) an investigation of the sensitivity of each parameter in the chemical model assumed; 3) an examination of the influence of  $S_{\text{int}}(A)$  on the accuracy and precision of the parameters estimated; and 4) for a given type of data and model expected in experimental spectra, to find the precision and accuracy of parametric values.

## Conclusion

Structural classification of equilibrium spectra-analyzing programs makes possible easier elucidation of sophisticated *modus operandi* and helps to understand the experimental and computational procedure of chemical model building and testing.

## SPECTROPHOTOMETRY *continued*

## References

- HAVEL, J. and MELOUN, M., in *Computational Methods for the Determination of Formation Constants* (Plenum Press, New York, 1985).
- MELOUN, M. and HAVEL, J., *Computation of Solution Equilibria. Part I. Spectrophotometry* (Folia Fac. Sci. Nat., Univ. Purkynianae Brunensis, Brno, 1986).
- HARTLEY, R., BURGESS, C., and ALCOCK, R.M., *Solution Equilibria* (Ellis Horwood, Chichester, 1980).
- MELOUN, M., HAVEL, J., and HÖGFELDT, E., *Computational Methods in Potentiometry and Spectrophotometry: A Guide to Present Day Methods in Equilibrium Analysis* (Ellis Horwood, Chichester, 1986) (in press).
- GAIZER, F., *Coord. Chem. Rev.* 27, 195 (1979).
- LEGGETT, D.J., *Am. Lab.* 14 (1), 29 (1982).
- SILLÉN, L.G. et al., *Acta Chem. Scand.* 16, 159 (1962); *Acta Chem. Scand.* 16, 173 (1962); *Acta Chem. Scand.* 18, 1085 (1964); *Arkiv Kemi* 23, 97 (1964); *Arkiv Kemi* 31, 315 (1968); *Arkiv Kemi* 31, 341 (1968); *Arkiv Kemi* 31, 353 (1968); *Arkiv Kemi* 31, 365 (1968).
- MELOUN, M. and ČERMÁK, J., *Talanta* 31, 947 (1984).
- MELOUN, M. and JAVŮREK, M., *Talanta* 32, 973 (1985).
- MELOUN, M., HAVEL, J., and JAVŮREK, M., *Talanta* 33 (1986) (in press).
- HIMMELBLAU, D.M., *Process Analysis by Statistical Methods* (John Wiley and Sons, New York, 1970).
- LUKŠAN, L., "SPONA-Set of programs for optimization and nonlinear approximation," Research Report No. V-4, (Czechoslovak Academy of Sciences, Praha, 1976).
- JAMES, F. and ROOS, M., *Comp. Physics Commun.* 10, 343 (1975).
- SILLÉN, L.G. and WARNQVIST, B., *Acta Chem. Scand.* 22, 3032 (1968).
- NAGYPÁL, I. and ZÉKÁNY, L., *Computational Methods for the Determination of Formation Constants*, D.J. Leggett, Ed. (Plenum Press, New York, 1985).
- VACCA, A., SABATINI, A., and GANS, P., *Talanta* 21, 53 (1974).
- PERRIN, D.D. and SAYCE, I.G., *Talanta* 14, 833 (1967).
- SAYCE, I.G., *Talanta* 15, 1397 (1968).
- SIMMONDS, J.L., *J. Opt. Soc. Amer.* 53, 968 (1963).
- WERNIMONT, G., *Anal. Chem.* 39, 554 (1967).
- WALLACE, R.M. and KATZ, S.K., *J. Phys. Chem.* 68, 3890 (1964).
- VARGA, L.P. and VEATCH, F.C., *Anal. Chem.* 39, 1101 (1967).
- KANKARE, J.J., *Anal. Chem.* 42, 1322 (1970).
- HAVEL, M. and MELOUN, M., *Talanta* 33 (1986) (in press).
- LEGGETT, D.J. et al., *Anal. Chem.* 47, 1065 (1975); *Anal. Chem.* 49, 276 (1977); *Talanta* 30, 579 (1983).
- NAGANO, K. and METZLER, D.E., *J. Am. Chem. Soc.* 89, 2891 (1967).
- REILLEY, C.N. and SMITH, E.M., *Anal. Chem.* 32, 1233 (1960).
- MELOUN, M. and JAVŮREK, M., *Talanta* 31, 1083 (1984).
- LIKUSSAR, W., *Anal. Chem.* 45, 1926 (1973).
- MELOUN, M. and ČERMÁK, J., *Talanta* 26, 569 (1979).
- MELOUN, M. and ČERMÁK, J., *Talanta* 23, 15 (1976).