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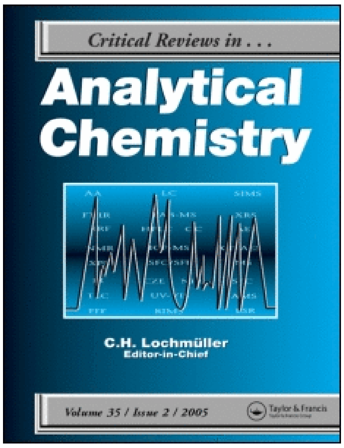

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# SPECTROPHOTOMETRIC METHODS FOR THE INVESTIGATION OF METAL COMPLEX FORMATION

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## I. INTRODUCTION

Significant analytical reactions of elemental species are usually based on complex formation during which various types of complexes, chelates, ternary species, and ion association complexes of suitable stability and analytical properties may be formed. Organic complex-forming agents often give species which highly absorb radiation in the visible (VIS) and UV spectral range and have been under the scope of analysts for many years. The development and studies of organic analytical reagents have stimulated the progress and achievements in both analytical and coordination chemistry.

A stepwise complex formation with complexing agents has been widely proved in water or polar mixed solvents.[1,2] Rather complicated equilibria may be established in aqueous and mixed solutions with mononuclear, oligonuclear, ternary, and ion association species of the analyte. These are then the basis of the particular detection and determination of an analyte, or the reason for competitions and interferences during practical analysis. In fact, more complicated complex equilibria were often discovered in aqueous solutions than had previously been suggested, but the same is true during extraction of an analyte species into the nonaqueous phase.

### A. The Role of Organic Analytical Reagents

Organic analytical reagents are inherently joined with the gravimetric and titrimetric procedures, solvent extraction, molecular absorption spectrophotometry in VIS and UV, or luminiscence. In addition, they may behave as suitable shifting reagents in nuclear magnetic resonance (NMR), surface modifiers and atomization enhancers in AAS, selective entrainers of trace elements, or important constituents of chelating sorbents. At present, complexing agents play a significant role in modern polarography and potentiometry including ISE, flow injection analysis, and various chromatographic techniques including high-pressure liquid chromatography (HPLC), or represent a substantial basis for postcolumn reaction detectors in connection with inorganic HPLC.[3]

### B. Complexation in Solution with Respect to the Optimization of Analytical Methods

Both coordination and analytical chemists are interested in the following: (1) prevailing equilibria in solution; (2) stoichiometry, stability, physical properties, and formation intervals of particular complexes; (3) structure, bonding sites, and the kind of bonding in particular complexes; (4) characteristic donor atom groups responsible for complex formation and analytical reactivity; and (5) exactly why some particular complexes are formed.

In addition, the analytical chemist wants to know (1) how to select conditions for the sole and quantitative formation of the most suitable complex used as the basis of a particular analytical method; (2) the reason, sensitivity, and selectivity of the analytical reaction based on complexation; and (3) the precision, repeatability, and accuracy of an analytical procedure based on complexation.

### C. Approach to the Studies of Complex Formation in Solution

The following points have to be respected: (1) have a pure and defined reagent or test its stoichiometry, ballast inerts, and impurities,[4] (2) evaluate acid-base equilibria of the reagent, (3) complex equilibria under broad experimental conditions (solutions containing excess of a particular reacting component or equimolar solutions); and (4) complex equilibria in the presence of a third component (buffer, additional complexing agent, masking agent, or surfactant) where ternary complexes, micellar complexes, or competition or substitution equilibria may be significant.

Spectrophotometric (i.e., VIS, UV), potentiometric, and polarographic methods, or solvent extraction under broad experimental conditions and with subsequent computer treatment of experimental data are very powerful methods for studying complex equilibria in solution. VIS and UV spectrophotometry enables the evaluation of all necessary parameters of complex equilibria in solution and has been most frequently used since most analytically important complexes in solution absorb radiation in the VIS or UV. The particular advantage of such investigation is in supplying parallel sets of data of various wavelengths spanning the whole absorption spectrum. Some disadvantage exists in the evaluation of two parameters always necessary for each complex species, i.e., the equilibrium or stability constant and the molar absorptivity for a selected set of wavelengths. If spectrophotometry is being used to study complex equilibria, some necessary conditions must be fulfilled: (1) high monochromacy of the radiation used, (2) observance of the Bouguer-Lambert-Beer law in the system under experimental conditions, (3) additivity of the absorbance function for all absorbing species present in the solution, and (4) satisfactory precision of absorbance measurements and no systematic instrumental or subjective error (bias).

An approved way to study complex equilibria in solution by using spectrophotometry in the VIS and UV range is summarized in Table 1. The graphical treatment of various absorbance plots is suitable for separated, distinguished, or successive complex formation intervals, but approximate or preliminary results are only obtained for complex mixtures or complicated equilibria. In such cases or for overlapping equilibria, a numerical analysis of spectrophotometric data may be successful using general minimization programs and high-efficiency computers. Several detailed reviews and discussions have already been published describing various up-to-date states of the art.[5-15]

The spectrophotometric investigation of chelates and complex ion pairs during solvent extraction requires some modification of approaches and care during evaluation of the experimental data.[16-19] Various kinds of spectrophotometric methods have been successfully applied to studies of ternary complexes containing two different ligands which may be formed from the corresponding binary species with or without partial substitution of the primary ligand. Such complexes show increased stability and outstanding optical properties. In such cases, the reactivity in binary systems should be previously explained and the measurements provided for a large interval of wavelengths and concentration of components.[20-27] A special kind of defined ternary ion association complex is formed from binary complexes of metal ions with complexing dye reagents such as hydroxytriphenylmethane dyes in the presence of cationic surfactants in submicellar concentrations which are characterized by considerably high molar absorptivities and large bathochromic shifts of their absorption maxima.[28-32]

## Table 1
## AN APPROVED WAY OF SPECTROPHOTOMETRICALLY STUDYING COMPLEX EQUILIBRIA

1. Examination of absorption spectra: evaluation of suitable wavelengths; the number of species; resolution and deconvolution of spectra via computer treatment; evaluation of $\lambda_{max}$ and the isosbestic points
2. Method of continuous variations (Job curves) under defined conditions and computer treatment of resulting plots
3. Combined graphical treatment of various absorbance plots by
   (a) Selected slope-intercept transformations of equilibrium and conditional stability constants; regression analysis of such transformations;
   (b) Modeling of normalized absorbance curves by means of computer to distinguish among equilibria tested;
   (c) Use of similar logarithmic transformations
4. Direct treatment of spectrophotometric data by means of computer using general minimization programs (for a large set of wavelengths, broad experimental conditions, and complicated mixtures of species)
5. Calculation of distribution diagrams and response surfaces for various species from evaluated parameters by means of computer; calculation of pure absorption spectra for particular species in mixtures

Experimental conditions: equimolar solutions; solutions with a metal ion or reagent excess; sufficient set of wavelengths; various kinds of continual techniques

To evaluate: the proper chemical model; the stoichiometry and number of species in solution; equilibrium, conditional, or true stability constants of species; spectral characteristics of species; optimum conditions for an exact spectrophotometric procedure for the determination of analyte

## II. BASIC EXAMINATION OF ABSORPTION SPECTRA IN THE VIS AND UV RANGE

Absorption spectra of analyte complexes and their changes in solution under broad experimental conditions (e.g., complexing agents or metal ions in variable excess and equimolar solutions at different pHs) give several pieces of qualitative information on the absorbing species, presuming that each absorbing species differs significantly in some part of the spectrum. This information is as follows: (1) identification of the number of absorbing species in solution (determination of the rank of the absorbance matrix) by using a computer; (2) interpretation of characteristic points on the absorption curves (absorption maxima and minima, inflection or isosbestic points); (3) selection of a suitable set of wavelengths for further investigation; (4) examination of the structural aspects from the spectra, including localization of the chromophore in the absorbing species, kind of metal-donor atom bonding, evidence of reagent tautomeres and particular structures of complexes in solution, and semiempirical quantum mechanical treatment of the reagent or metal complex species in order to explain the $\pi$ electron density on selected atoms, the nature and origin of the liberated protons, and the involved donor atom group.[33-35]

Some comprehensive literature is available on the explanation or discussion of absorption spectra of complexes in the VIS and UV range.[36-38] Highly absorbing chelates, ternary and ion association, or electron donator-acceptor complexes with organic analytical reagents show considerable color contrast and may be successfully investigated spectrophotometrically. Electron transfers between reagent and metal ion orbitals, back coordination along the metal-donor atoms bonding, delocalization of electrons during complexation, extension of $\pi$-electron resonance, or dyes with characteristic functional groups of donor atoms as involve most characteristic changes in absorption spectra in the visible region. The changes in complex stoichiometry, the successive reagent coordination to the metal or nonmetal species, the protonation or deprotonation of complex species or reacting components, as well as changes in properties of the solvent often influence the absorptivity and cause bathochromic or hypsochromic shifts of the absorption maximum. Absorption spectra are

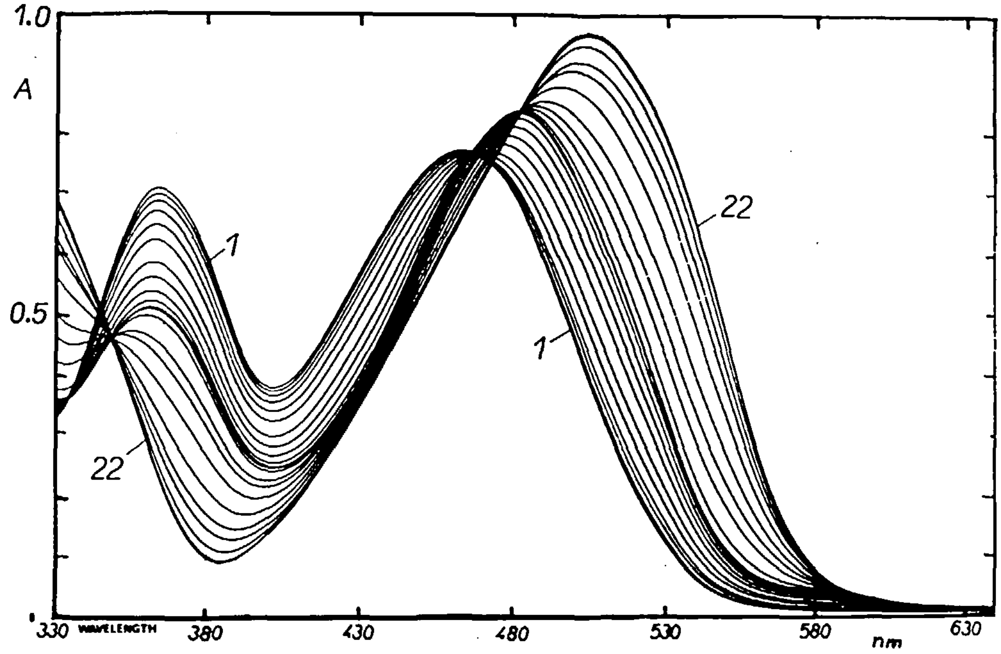

FIGURE 1. Absorption curves of 1-(2-pyridylazo)-2-naphthol-6-sulfonic acid vs. pH. Curves 1 to 12 correspond with a pH of 1.3 to 9.9 continuously increasing; $c_L = 4.4 \times 10^{-5}$ mol/ℓ. Isosbestic points characterize the transitions between $H_2L^+ \rightarrow HL$ (isosbestic points, 335 and 468 nm) and $HL \rightarrow L^-$ (isosbestic points, 349 and 482 nm).

usually recorded for solutions with metal ion or ligand excesses or for equimolar solutions, in dependence on the pH.

Isosbestic points on absorption curves give evidence about defined stoichiometric reactions between components and separated complex formation steps in the absence of side reactions, or about kinetically different steps. The number of isosbestic points is not simply related to the number of complexation steps. Each extremum point on the curve essentially has its own isosbestic point. Stationary equilibria or slow kinetic processes can show isosbestic points, for which $(\partial A/\partial pH)\lambda_{iso}$ or $(\partial A/\partial p)\lambda_{iso} = 0$, where $p = c_L/c_M$.

Thus, an isosbestic point may characterize the following cases:

1. $\epsilon_{k1} = \epsilon_{k2}$: transition between two absorbing complexes or two different ligand species
2. $n\epsilon_L = \epsilon_{k1}$: the absorbing ligand is bound into an absorbing $M_mL_n$ complex
3. $m\epsilon_M = \epsilon_{k1}$: the absorbing metal ion is bound into an absorbing $M_mL_n$ complex

$\beta_i$ of the complex equilibrium may simply be calculated from $\epsilon$ of the isosbestic point and the absorbance curve before passing the point for the same wavelength.[39,40] For illustration, Figures 1, 2, and 3 are shown.

Approximate graphical deconvolution procedures of composed absorption spectra into simple Gaussian maxima are available to reveal the number of absorbing complexes or structural or tautomeric reagent forms of the reagent in solution.[41,42] A different graphical way was described for the evaluation of the number of species from the spectrum based on linearization of some derived absorbance functions.[43]

A detailed and exact interpretation of absorption spectra may be carried out by the matrix analysis of spectra for solutions with various pHs or concentrations of components, assuming that the Bouguer-Lambert-Beer law and the additivity of absorbances are closely obeyed.

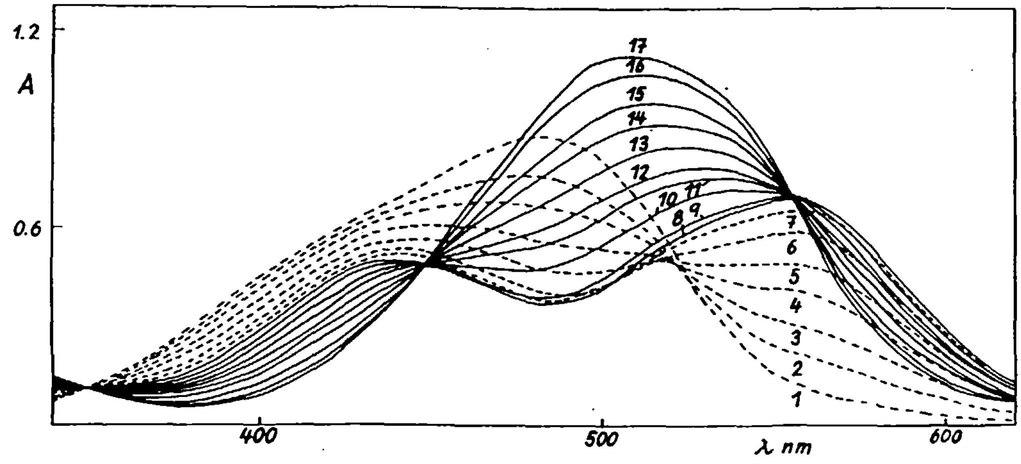

FIGURE 2. Absorption curves in the system $Cu^{2+}$- 4-(2-thiazolylazo)resorcinol in the presence of metal ion excess for various pHs. $c_L = 3.7 \times 10^{-5}$ mol/ℓ, $c_M = 2.9 \times 10^{-3}$ mol/ℓ; isosbestic point, 520 ($LH_3^+ \rightarrow CuLH$; pH 0.4 to 2.0), 555, and 448 nm ($CuLH \rightarrow CuL$; pH 2.1 to 6.1).

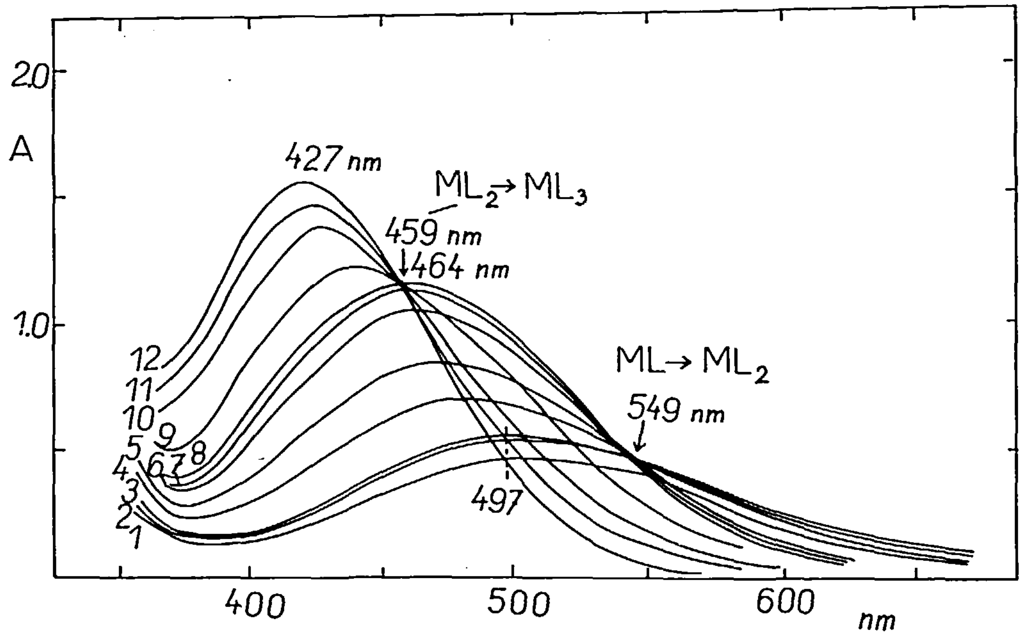

FIGURE 3. Absorption spectra in the system Fe(III)-5-sulfosalicylic acid in solution with reagent excess for various pHs. $c_M = 2.8 \times 10^{-4}$ mol/ℓ, $c_L = 7.1 \times 10^{-2}$ mol/ℓ; isosbestic point, 549 nm ($ML \rightarrow ML_2$; pH 1.6 to 4.0), isosbestic point, 460 nm ($ML_2 \rightarrow ML_3$; pH 4.5 to 9.0).

This may be formally expressed by Equation 1:

$$A_{ij} = \sum_{k=1}^{M} \epsilon_{ik} c_{kj} \quad \text{or} \quad A = EC \qquad (1)$$

where $i\epsilon\{1\ldots I\}$, $j\epsilon\{1\ldots N\}$, $k\epsilon\{1\ldots M\}$, A is the absorbance matrix of the type I/N, E is the matrix of molar absorptivities of the type I/M, C is the concentration matrix of the type M/N, N is the number of solutions, M is the number of absorbing species, and I is the number of wavelengths. The rank of the matrix A defines the number of nonzero elements on the matrix diagonal and corresponds to the number of absorbing species in the system. The rank of the matrix is calculated according to various procedures, e.g., the triangulation of the absorbance matrix and its comparison with the error matrix treated in the same way.[44-46] A different procedure is also used on the calculation of the second matrix moment and the analysis of the particular eigenvalues or eigenvectors by factor analysis.[48] Because of random errors in absorbance data, the major problem of matrix analysis of the absorption spectra is to select effective statistical criteria for deciding elements reaching zero values.[14] For experimental testing, wavelengths are chosen having the most distinct changes in the spectrum, and solutions are examined covering a broad interval of pHs and concentrations of components. Other simpler mathematical procedures have also been tested for the deconvolution of spectra to individual Gaussian components and the determination of the number of absorption maxima belonging to the individual components in the spectrum.[49-51]

## III. SPECTROPHOTOMETRIC VARIANT OF THE METHOD OF CONTINUOUS VARIATIONS

The simple variant of the method developed by Ostromyslenskii,[52] Denisson,[53] Job[54] and Vosburgh and Cooper[55] gives absorbance plots against the molar fraction of a ligand or metal ion, $x_L = c_L/(c_M + c_L)$ or $x_M = c_M/(c_M + c_L)$, maintaining the $c_M + c_L = c_0$ constant in the whole series of solutions. This may be realized by complementary volumes of equimolar solutions at the constant total volume. Considering a single defined equilibrium:

$$mM + nL \rightleftarrows M_mL_n \tag{A}$$

$\epsilon_L = \epsilon_M = 0$; and $x_M$, $x_L$, $v_M$, and $v_L$ are the particular molar fractions or volume of equimolar solutions, respectively; the coordinates of the maximum of the plot $A = f(x_M$ or $x_L)$ or $A = f(v_M$ or $v_L)$ may give the stoichiometry of the complex being formed in solution:

$$n/m = \frac{x_{L(max)}}{1 - x_{L(max)}} = \frac{1 - x_{M(max)}}{x_{M(max)}} \tag{2}$$

If the reactant absorbs radiation at the wavelengths used, the following function Y is to be plotted:

$$Y = \epsilon_1 c_1 + \epsilon_2 c_2 + \epsilon_3 c_3 - \epsilon_1 c_0(1 - x_L) - \epsilon_2 c_0 x = A - A_0 \tag{3}$$

where $A_0$ is the sum of absorbances of reacting components prior to the reaction. The condition of maximum on the Job plot is then defined:

$$\frac{dY}{dx} = \frac{dc_3}{dx}(\epsilon_3 - m\epsilon_1 - n\epsilon_2) \tag{4}$$

where $c_1$, $c_2$, and $c_3$ are concentrations of the metal ion, reagent, and complex, respectively. For $\epsilon_3 > m\epsilon_1 - n\epsilon_2$, there is a maximum on the Job plot. On the other hand, errors in evaluation of stoichiometric coefficients result if A is plotted instead of Y and $\epsilon_1 \neq \epsilon_2$.[56] A true $Y = f(x_{L(M)})$ plot can simply be realized graphically by subtracting the absorbance along a connecting line between the extremum points for $x_L = 0$ ($A_{01} = \epsilon_M c_0$) and $x_L = 1$ ($A_{02} = \epsilon_L c_0$) from the actual absorbance plot, $A = f(x_L)$. The true position of the maximum on this plot may then be indicated by a tangent line parallel to the connecting line from $x_L = 0$ to $x_L = 1$.[57] The graphical evaluation of complex stoichiometry is inaccurate for complexes with $n/m = x_L/(1 - x_L) > 3$ since a 2% relative error in the preparation of the solutions or reading the coordinates on the Job plot changes the value of n/m by 1. In addition, the evaluation of the maximum is rather difficult for a very unstable complex with n/m > 1.

There are serious limitations for using the method of continuous variations for the evaluation of complex stoichiometry and of basic complex parameters:

1. A single complex equilibrium must be established in solution.[11,56,58-61]
2. Additional successively formed absorbing complexes may sometimes be detected or eliminated by measuring at a suitably chosen set of wavelengths.[62]
3. Monomeres and oligomeres having the same component ratio may be distinguished only with difficulty.
4. No competing reaction equilibria with the free reacting components take place.
5. Constant ionic strength and constant pH should be maintained for all solutions and no buffer used if possible.
6. Conditional values of stability constant and molar absorptivity of the complex usually result from the interpretation of Job plots.

For some more complicated equilibria, the parameters of the maximum give the stoichiometric coefficients of the reaction components formed rather than their molar ratio in the complex formed.[61-63] For the following reaction only,

$$A + nB \rightleftarrows AB'_n + cC + dD + \ldots \qquad \text{(B)}$$

where B′ is a part of B, the position of the $x_{max}$ is not influenced.[64] Polymerized components may cause interferences during the evaluation of the maximum of the Job curve if they depolymerize to optically different monomeres when being complexed.[64] Under special conditions, the stoichiometry can be evaluated even in the presence of competing equilibria; nevertheless, hard restrictions must be respected for the calculation of the stability constant and molar absorption coefficients. Several more complicated cases have been discussed.[52,54,55,63,67]

Job curves for systems requiring constant pH of solutions in the absence of any buffer may be interpolated graphically as perpendicular parallels from a set of Y = f(pH) plots for all particular $x_L$ or $x_M$, respectively. Besides the traditional experimental flask (batch) procedure, points may be obtained from a single titration[68] or by continual mixing of component solutions from burettes which produce a large continuous set of experimental points.[14]

Job curves represent hyperboles of various order dependent on the stability and the stoichiometry of the complex formed as well as on the concentration of components $c_0 = c_M + c_L$. They may degenerate in a pair of straight lines crossing in the maximum for the conditional stability of $\beta_i \rightarrow \infty$. According to practical experience, the actual abscissa of the maximum of the Job curve corresponds better to the molar ratio of the components in the complex than the intersection points of the tangents to the curve at $x_L = 0$ and $x_L = 1$, especially for unstable 1:1 complexes or when asymmetric Job curves belonging to a stable complex are evaluated. The theoretical shapes of normalized Job curves, the stoichiometry of the complex being formed, and the slopes of the curves in the limiting points $x_{L(M)} = 0$ and $x_{L(M)} = 1$ were explained by various computational approaches.[69-77] The experimental Job curve is transformed to the normalized one with respect to the following:

$$M_mL_n = \frac{A - A_L - A_M}{\epsilon_k - m\epsilon_M - n\epsilon_L} \qquad (5)$$

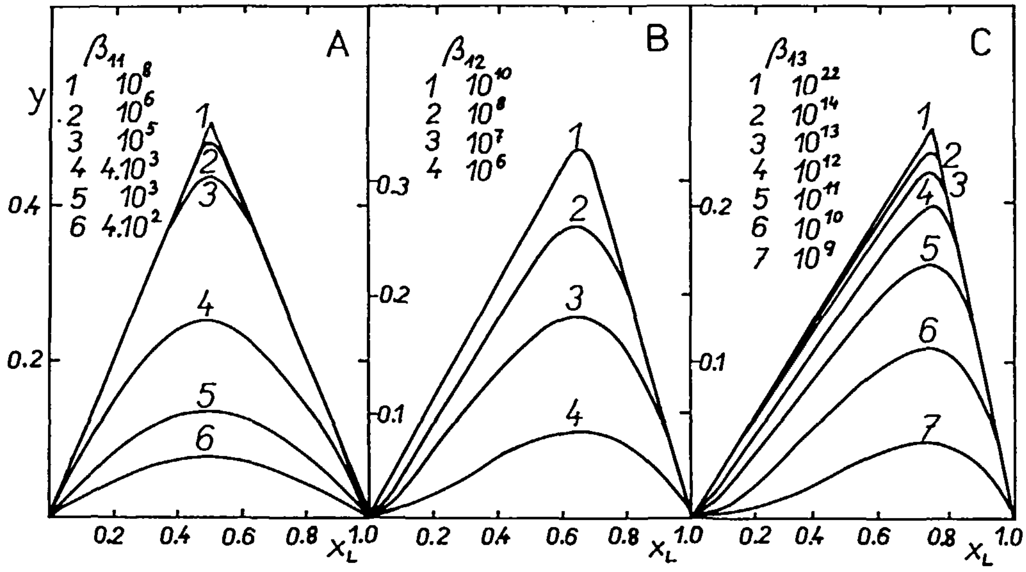

FIGURE 4. Computed normalized Job curves for complexes of various stoichiometries dependent on their stability. (A) Formation of ML; (B) formation of $ML_2$; (C) formation of $ML_3$; $c_0 = 10^{-3}$ mol/ℓ.

$$(1 - x_L - my)^m (x_L - ny)^n - (1/\beta_{mn} c^{m+n-1}) y = 0 \tag{6}$$

where $y = [M_m L_n]/c_0$.

Thus, Job curves for various stoichiometry and assumed stability constants of the complex formed were simulated by using the HALTAFALL-SPEFO program.[77] Approximately 40 points of such curves were used for computation by means of a computer, and the resulting curves were drawn and compared (see Figure 4).

### A. Complex Formation of Various Stoichiometries

The formation of a ML complex results in symmetrical curves, but for sufficiently stable complexes only (e.g., $\beta'_1 > 10^5$), there is an intersection of limiting slopes at $y_{max}$ and $x_{max} = 0.5$, corresponding to the quantitative complex formation and $[ML] = c_0/2$. For complexes with $\beta'_1 < 10^5$:

$$(dy/dx_L)_{x_L=0;\ x_L=1} = \pm\frac{\beta'_1 c_0^2}{\beta'_1 c_0 + 1} \tag{7}$$

$$(dY/dx_L)_{x_L=0;\ x_L=1} = (\epsilon_k - \epsilon_M - \epsilon_L)\frac{\beta'_1 c_0^2}{\beta'_1 c_0 + 1} \tag{8}$$

which is simplified for $\beta'_1 c_0 \gtrsim 10^2$:

$$(\epsilon_k - \epsilon_M - \epsilon_L) c_0 = \pm(dY/dx_L)_{x_L=0;\ x_L=1} \tag{9}$$

Curves related to $M_m L_n$ complexes of medium stability indicate inflection points or concave curvatures near the limiting points of the curve:

1. Left side inflection point ($x_L \rightarrow 0$): $n > 1$; $m = 1$
2. Right side inflection point ($x_L \rightarrow 1$): $n = 1$; $m > 1$
3. Both sides inflection points: $n > 1$; $m > 1$

Slopes $(dy/dx_L)_{x=0}$ or $-(dy/dx_L)_{x=1}$ then reach zero for the concavely deformed ends of the curve,[6,11,70,72] but the curvature is only evident on the curve for complexes of low and medium stability at small $c_0$ values. From some particular values of these parameters, i.e., from $\beta'_{22} > 10^{10}$ for $M_2L_2$ complex and $\beta'_{23} > 10^{15}$ for $M_2L_3$ complex referring to $c_0 = 10^{-3}$ mol/ℓ, the curves are fully convectional, and their branches change into two straight lines with an intersection point. This fact may explain the early disagreement among various authors.[70-73] Thus, the extrapolation of limiting tangent lines to evaluate complex stoichiometry and parameters may be wrong for nonsymmetrical Job curves except complexes $M_mL_n$ with very high values of condition stability constants which are $\beta'_2 \geqslant 10^{10}$ (formation of a $ML_2$ complex) or $\beta'_3 \geqslant 10^{20}$ (formation of a $ML_3$ complex) and $c_0 = 10^{-3}$ mol/ℓ.[78] Omitting the mathematical sense, the values of both limiting slopes of the $Y = f(x_L)$ plots for the formation of a very stable $M_mL_n$ complex are defined as follows:

$$-(dY/dx_L)_{x_L=1} = \frac{n}{m}(\epsilon_k - n\epsilon_L - m\epsilon_M)c_0 \tag{10}$$

$$+(dY/dx_L)_{x_L=0} = \frac{m}{n}(\epsilon_k - n\epsilon_L - m\epsilon_M)c_0 \tag{11}$$

For the medium stable complexes $ML_n$ (with $n > 1$), the derived slopes for the particular Job curve have the following forms for the real limit ($x_L = 1$):

$$-(dY/dx_L)_{x_L=1} = \frac{\beta'_n c_0^{n+1}}{\beta'_n c_0^n + 1}(\epsilon_k - \epsilon_M - n\epsilon_L) \tag{12}$$

which simplifies for $\beta'_n c_0^n > 10^2$ to

$$-(dY/dx)_{x=1} = c_0(\epsilon_k - \epsilon_M - n\epsilon_L) \tag{13}$$

A procedure was developed to evaluate stoichiometric coefficients m and n in a dissociated $M_mL_n$ complex from $x_L$ abscissae near the limiting points.[79] The exponential expressions in the general equation of the normalized Job plot were treated by Taylor expansions separately, simplified for $x_L \to 0$ or $x_L \to 1$, logarithmed, and derived to limits:

$$\lim_{x_L \to 0} \frac{\partial \log y}{\partial \log x_L} = n \tag{14}$$

$$\lim_{x_L \to 1} \frac{\partial \log y}{\partial \log(1 - x_L)} = m \tag{15}$$

which may be interpreted graphically

$$\frac{\Delta \log y}{\Delta \log x_L} = f(x_L) \quad \text{or} \quad \frac{\Delta \log y}{\Delta \log(1 - x_L)} = f(x_L) \tag{16}$$

The same simplified expressions enabled the calculation of the conditional stability constant.

Procedures for distinguishing complexes of various stoichiometry, especially complexes ML and $M_2L_2$, were described by (1) comparing shapes of normalized Job curves near the limiting points of $x_L$,[74,79-81] a procedure that is seriously limited by the stability of particular complexes (e.g., $\beta'_{22} \leqslant 5.10^9$ for $c_0 = 10^{-3}$ mol/ℓ; see Figure 5); (2) calculation procedures

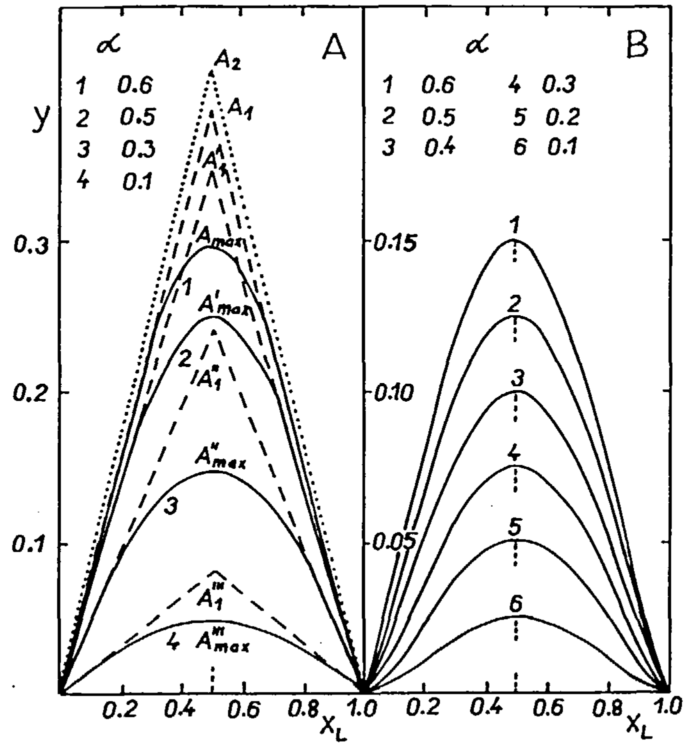

FIGURE 5. Different shapes of Job curves for (A) the ML complex and the (B) $M_2L_2$ complex, with respect to various stabilities or different dissociation degrees $\alpha = (m + n)[\text{Complex}]/c_0$, $c_0 = 10^{-3}$ mol/ℓ ($0 > \alpha > 1$), for both complexes. $A_{max}$ (absorbance in the maximum of the Job plot); $A_1$ (extrapolated absorbance from the limiting $x_L = 0$ and $x_L = 1$); $A_2$ quantitative complex formation which plays its role in the determination of the molar absorptivity and the conditional stability constant from the limiting points of the Job plots.

related to normalized experimental Job curves;[82-84] and (3) the graphical linearization of some part of the Job curve or the interpretation of a plot $Y_{max}$ vs. $c_0$ from Job curves for various $c_0$ values according to a complicated equation.[82] A dependence $Y_m^{(1-m-n)/(m+n)}$ vs. $c_0/(m + n)Y_{max}$ is linear for correct values of m and n, but ambiguous results are obtained, especially for $m + n \geq 5$.

## B. Evaluation of Conditional Stability Constants and Molar Absorptivities by the Job Curves

There were a number of early attempts to evaluate conditional stability constants and molar absorptivities from the parameters of the Job curve maximum, from the slopes at the limiting points of the curve or at other points belonging to a single or two Job curves for various $c_0$, from the limiting slopes of two Job curves for various $c_0$, etc., but their significance has been lost because more suitable and accurate spectrophotometric procedures are now available. They were summarized in earlier reviews.[6,9,57,69,70,82-93] Procedures exploiting slopes in limiting points of the Job curve give unsatisfactory results for weak complexes;[69,70,78] molar absorptivities usually follow only from Job curves for very stable complexes. Some problems with the evaluation of stoichiometry of complexes with decreasing stability, the limiting slopes of the Job curves under various conditions, and the determination of conditional constants from two points of such curves have been discussed.[94]

### 1. *Evaluation of Conditional Stability Constants from $A_{max}$ of the Job Plots for Solutions with Various $c_0 = c_M + c_L$*

This was realized from a complicated equation:

$$\frac{c_0}{(m + n)Y_{max}} = Y_{max}^{(1-n-m)/(n+m)}[n^n m^m \beta'(\epsilon_k - n\epsilon_L)]^{-1/(n+m)} + \frac{1}{(\epsilon_k - n\epsilon_L)} \tag{17}$$

If the true m and n coefficients (evaluated from the coordinates of the maximum) are substituted in Equation 17, the corresponding plot is linear, and the molar absorptivity is calculated from the intercept on the y coordinate and the conditional stability constant from the slope. On the other hand, the stoichiometric coefficients n and m of the complex $M_mL_n$ may be tested or distinguished by the straight-line course of the plot.[82]

### 2. *Calculation of the Conditional Stability Constant from the Maximum of the Job Curve Only, Where the Influence of Possible Competing Equilibria in Limiting Values of $x_L$ or $x_M$ Are Eliminated*

The stoichiometry of the complex must first be evaluated, and the stability constant is determined graphically combining the general normalized Equation 6 and additional plots of various absorbance ratios $A_{(c0)}/A_{(c0)}$ related to successively diluted solutions of the complex with $c_0/(c_0/q)$ in dependence on log $(\beta' c_0^{m+n-1})$; q is the dilution factor. Since the sets of such plots differ for complexes of various stoichiometry, the procedure may distinguish between complexes of various stoichiometry.[73,74,95] A similar procedure has also been described.[80]

### 3. *Calculation of the Constant and Molar Absorptivity of a 1:1 Complex of Limited Stability from the Slopes in the Limiting Points $x_{L(M)} = 0$ and $x_{L(M)} = 1$ and the Absorbances in the Maximum of the Symmetrical Experimental Job Curve ($A_{max}$ and $A_1$)*

The intersection of extrapolated tangents defines a parameter d:

$$d = (A_1 - A_{max})/A_1 \tag{18}$$

(See Figure 5.)

The following equation results with respect to the equation for the maximum of the Job curve:

$$d = \frac{1}{(\beta' c_0)^2}[(1 + 2\beta' c_0)^{1/2}(1 + \beta' c_0) - 2\beta' c_0 - 1] \tag{19}$$

A tentative function d = f($\beta' c_0$) is then plotted for various assumed $\beta' c_0$ values, and the true condition constant is evaluated from the graph from the identical particular d value. Such a procedure gives correct values of conditional stability constants, unlike some earlier procedures.[96] The accuracy of evaluation of the constant depends on the correctness for the extrapolation of the limiting tangents. The molar absorptivity is calculated from the particular apparent value $\epsilon_{app}$:

$$\epsilon_{app} = 2A_1/c_0 \tag{20}$$

$$\epsilon_{app}/\epsilon = \beta c_0/(\beta c_0 + 1) \tag{21}$$

and both the stability constant and the molar absorptivity are proved by a derived logarithmic plot of the Job curve.[78]

### *4. The Least-Squares Procedure by Means of JOBCON Program*[83,84]

For the evaluation of the conditional stability constant and the stoichiometry of the complex, the procedure is applicable for aqueous solutions and during liquid-liquid extraction. A normalized absorbance scale was expressed by

$$y = [M_mL_n]/[M_mL_n]_{max} = A/A_{max} \tag{22}$$

where $A_{max}$ was related to $c_M$ corresponding with the quantitative formation of the complex in the maximum of the Job curve. It is realized for a solution with a large excess of reagent (the absorbance of the blank subtracted) or explicitly evaluated by successive approximation according to the program. The following equations have been interpreted:

$$\beta'_{mn} = [(m + n)/c_0]^{m+n-1}y[(m + n)x - my]^{-m}[(m + n)(1 - x)ny]^{-n} \tag{23}$$

$$[(m + n)x - my]^m[(m + n)(1 - x) - ny]^n - ym^mn^n(1 - y_{max})^{m+n}/y_{max} = 0 \tag{24}$$

The conditional stability or extraction constant was calculated from the average value of $\bar{y}_{max}$ obtained from various $y_{max}$ values resulting for particular points of the curve:

$$\bar{y}_{max} = \sum_{i=1}^{n} y_{max}/n \tag{25}$$

where n is the number of points on the Job curve. In the original, the computer tests 15 different complexation models according to the program for various m:n = 1:1 − 1:8 and 2:1 − 8:1 with respect to the constant value of the conditional stability constant for the extremal point of the curve. The minimum value of the relative standard deviation $s_{rel}(\beta')$ resulting from $\bar{y}_{max} - y_{i(max)}$ is taken as the criterion for the true model. Optimum intervals for $A = 0.1 - 1.0$ or $y_{max} = 0.20 - 0.95$ were recommended for interpretation. Such a procedure is, of course, worthy only if a single complex is formed in solution during experimental conditions. The number of absorbing species formed during the continuous variations may be determined by the Simmond-Vernimont-Kankare method.[97,98]

Summarizing computations within continuous variation curves is relatively easy if the complex being formed is of medium stability. The computation may, however, fail if the complex is either extremely stable or very weak.

## C. Job Curves during Solvent Extraction

Aqueous solutions of equimolar reacting components are mixed, and the complex being formed is extracted into a constant volume of nonaqueous phase from a constant total volume of aqueous solution. The stoichiometry of the extracted complex corresponds to the coordinates of the maximum of the Job curve.[99,100] The solvatation number of the analyte complex may also be determined if a constant aqueous volume of increasing molar fraction of the metal ion and a constant excess, especially a small excess, of reagent is mixed with a constant volume of organic phase containing increasing volumes of active solvent in the inactive solvent.[101-109] It is not recommended to mix equimolar aqueous metal ion solutions with the complementary equimolar solution of the reagent in the organic solvent (i.e., volume of theorganic phase not constant during the series of solutions) since the coordinates of the Job maximum may differ from the real stoichiometry of the studied complex in the organic phase.[99,104] The stoichiometry of the extracted species is not influenced in the case of stepwise formation of complexes in aqueous solution.[105]

### D. Formation of Ternary Complexes

Ternary complexes with different ligands L and X may be formed according to general equilibria

$$M + nL + pX \rightleftarrows ML_nX_p \tag{C}$$

or

$$ML_n + pX \rightleftarrows ML_{n-p}X_p + pL \tag{D}$$

The stoichiometry and the properties of the ternary complex formed, in particular the molar absorptivity and the conditional stability constant of the complex, decide the kind of shift of the Job maximum.[90] Two procedures are used applying the method of continuous variations:

1. Equimolar solutions of M and L or M and X are successively mixed in the complementary ratio at a constant and small excess of the third component; deviations between coordinates of the maximum of the Job curve and the real stoichiometry of the ternary species may be found if substitution equilibria are involved. Various constant concentration excesses of the third component should be tested.
2. The absorbance of equimolar solutions of all three components M, L, and X are mixed in such a way that the resulting volume of all components remains constant ($c_M + c_L + c_X = c_0$). The apex of so-called isochromates in an equilateral triangle determines the stoichiometry of the ternary complex. Wavelengths for measurements at which the molar absorptivity of the ternary species is sufficiently different from that of the binary complex must be carefully selected. Such a procedure is time consuming because of preparation and measurement of many solutions.[5,106-108] If some ligand substitutions are suspected during the formation of ternary species, the concentration of reacting components must be tested in broad concentration intervals for several wavelengths, and the resulting position of the maximum of the Job curve compared.[109-111] The competition of two metal ions with a particular ligand has been commented on.[90]

### E. Continuous Variations in Nonequimolar Solutions

Solutions of metal ion are mixed with those containing a variable constant excess of reagent, $p = c_L/c_M = 2 - 2000$ maintaining $c_0 = c_L + c_M$ constant in the whole series of solutions. The maximum of the Job curve is now regularly shifted to the left (i.e., lower values of $x_L$), and its coordinates are not identical with the stoichiometry of the complex formed. Such modification of the traditional procedure is suitable for studying systems with rather unstable complexes or easily hydrolyzable species. Successive equilibria are then shifted to the formation of the higher complex in the series. For the formation of a typical mononuclear complex $ML_n$, Equation 26 results:

$$\beta'_n = \frac{(p - 1)^n[n - x_{max}(1 + n)]}{[x_{max}(n + p) - n]^{n+1}p^{n-1}c_0^n} \tag{26}$$

The parameters of the maximum are useful for the calculation of the conditional stability constant, and its constant value for a particular tested n and curves with various p is an indirect proof for the stoichiometry of the complex formed in solution. Introducing small integers for n in Equation 26, the calculated conditional stability constant $\beta'_n$ maintains its constant value for various $p = c_L/c_M$ for the true n value only. Similarly, the relative error $d\beta'_n/\beta'_n$ for various p reaches the smallest value for the true n in the complex $ML_n$. On the other hand, the constant value of $\beta'_n$ is a simple proof that one complex is formed only under the working conditions.[91] The term in brackets of the determinator of Equation 26 nears zero for very stable complexes ($\beta'_n \rightarrow \infty$), and the coordinate of the maximum corresponds with Equation 28:[86,91]

$$(p + n)x_{max} - n \cong 0 \qquad (27)$$

$$x_{max} = \frac{n}{n + p} \qquad (28)$$

For very weak complexes, the calculation of the conditional stability constant is realized from pairs of points $x_{L1}$ and $x_{L2}$ on the Job curve of particular $c_0$ and p values for which the absorbance difference Y is identical.[9,112]

### F. Successive Complex Formation (Several Complexes Simultaneously Present in Solution)

If several complexes are simultaneously formed with a particular reagent, the shape and the coordinates of the maximum of the Job curve depend on the stoichiometry of the complexes present, their conditional stability constants, and their molar absorptivities, i.e., on the wavelength used for the measurements. Thus, some particular complex may be completely omitted, as may be shown by comparing calculated distribution plots, absorption spectra, and Job curves for the selected case of complexation.[113] A regular sequence of molar absorptivities $\epsilon_1$, $\epsilon_2$, $\epsilon_3$ often follows for three complexes with successively increasing ligands in the complex molecule, and the $x_{max}$ of the Job curves maintains an intermediate position between stoichiometric coefficients in dependence on the wavelength used for measurement. In such cases, the concentration maxima for the particular complex on the Job plot do not agree with the absorbance or Y maxima on the plot. This problem has been discussed by several authors.[9,55,77,114,115] Full absorption curves should be recorded first for various pHs and $c_0$ to select the most suitable wavelengths for individual complexes.[116] The position of the Job curve maximum is, however, influenced whether or not the accompanying complexes absorb at the wavelengths used for measurements. Fortunately, the stepwise-formed metal chelates with organic reagents often show considerable differences in optical properties and stabilities, so that particular steps can be distinguished by Job curves for various wavelengths, pHs, and $c_0$, or in the presence of a nonaqueous solvent. A large shift of the maximum of the Job curve indicates an erroneous stoichiometry for the last strongly absorbing complex ($n_{found} > n_{true}$).[75] Thus, no reliable information results about the stoichiometry of absorbing complexes from the Job maximum even if it is measured at the wavelength where accompanying complexes do not absorb.

### G. Effect of Secondary and Competing Equilibria on the Job Curve

The effect may be followed or tested by computer calculations by using the HALTAFALL SPEFO program[77] and modeling theoretical Job curves with respect to the analyte and interferent complexes of different stoichiometry and stability. Some particular cases of competition equilibria are summarized in Table 2 and Figure 6.

#### *1. Competition between Two Ligands*[77,90]

The shape of the studied analyte complex is deformed and the maximum of the plot shifted to lower $x_L$ values in the presence of a further nonabsorbing competing reagent X:

$$ML_n + pX \rightleftarrows MX_p + nL \qquad (E)$$

## Table 2
## COMPETING EQUILIBRIA AND COMPLEX STOICHIOMETRY

| Equilibrium | Apparent changes in complex stoichiometry[a] | | Ref. |
|---|---|---|---|
| Stepwise complex formation | $n_f < n_{th}$ | for $\epsilon_{n+k} = 0$ | 55 |
| $ML_n$, $ML_{n+k}$ | $(n + k)_f > (n + k)_{th}$ | for $\epsilon_n = 0$ | |
| Stepwise complex formation | $n_f < n_{th}$ | for $\epsilon_{n+k} = \epsilon_{n+k+q}$ | 114 |
| $ML_n$, $ML_{n+k}$, $M_{n+k+q}$ | $(n + k)_f > (n + k)_{th}$ | for $\epsilon_n = \epsilon_{n+k+q}$ | |
| | $(n + k + q)_f >$ $(n + k + q)_{th}$ | for $\epsilon_n = \epsilon_{n+k}$ | |
| $\Sigma_{i=1}^{n} ML_i$ $(n \geq 3)$ | $n_{i(max)f} \gg n_{i(max)th}$ | | 75 |
| Competition of two ligands with metal ion | $n_f = n_{th}$ | for $\epsilon_1 = \epsilon_2$ | 62, 90 |
| $M + nL \rightleftarrows ML_n$ $(\epsilon_1)$ | $n_f < n_{th}$ | for $p = 1$, $\epsilon_2 = 0$ | |
| $M + pR \rightleftarrows MR_p$ $(\epsilon_2)$ | $n_f = n_{th}$ | for an unstable complex $MR_p$, i.e., $c_R \gg p[MR_p]$ | |
| $c_R$ in constant excess | | | |
| Substitution equilibrium | $n_f > n_{th}$; | free metal ion absent in solution | 62 |
| $a\,ML_n + pR \rightleftarrows M_aR_p + an\,L$ | | | |
| Competition of two metal ions with a ligand | $n_f > n_{th}$ | for $\beta'(p - 1) < [L]^p$ | 90 |
| $M + nL \rightleftarrows ML_n$ $(\epsilon_1)$ | $n_f = n_{th}$ | for $\beta'(p - 1) = [L]^p$ | |
| $M' + pL \rightleftarrows M'L_p$ $(\epsilon_2)$ | $n_f < n_{th}$ | for $\beta'(p - 1) > [L]^p$ | |
| $c'_M$ in constant excess, $\epsilon_2 = 0$ | | | |
| Metal ion hydrolysis to oligonuclear species[b] | $n_f > n_{th}$ | | 90, 91, 205 |
| Protonation and deprotonation in nonbuffered solutions[c] | $n_f = n_{th}$ | for $[H] + [HL] = c_0x_L + s$ | 70 |
| | $n_f > n_{th}$ | for $[H] + [HL] = s$ | |
| | $n_f < n_{th}$ | for $[H] + [HL] = c_0x_L$ | |
| | | s, constant conc. of strong acid | |

[a] Related to Job curves with A(Y) vs. $x_L$ and resulting in shifts of $x_{L(max)}$.

[b] The shift of $x_{L(max)}$ to the right is time dependent; deformations and sometimes no maximum appear on the curve.

[c] In nonbuffered solutions, the $x_{L(max)}$ depends on the actual total proton activity, i.e., on the concentration and the dissociation (or protonation) constant of the ligand as weak acid or strong conjugated base.[70]

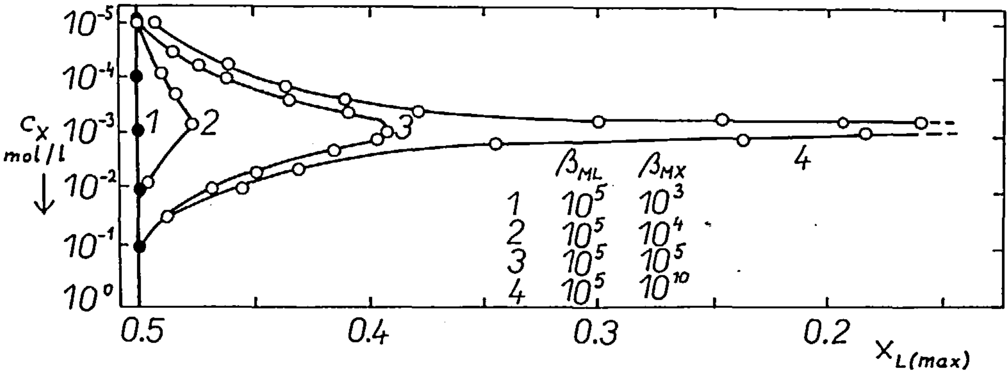

FIGURE 6. Shift of the maximum of the Job curve as a result of the competition between two complexing agent, L and X, dependent on the concentration of ligand X; formation of ML and MX complexes.

The influence of the competing agent (buffer, masking agent) depends on the conditional stability constants of both species, $ML_n$ and $MX_p$, and their stoichiometries. This makes the evaluation of the maximum for the analyte complex stoichiometry impossible.[90] On the other hand, the new position of the $x_{L(max)}$ of the leading analyte complex calculated for variable competing ligand excesses and particular stoichiometries under simplified conditions was compared to that of the experimental curves.[77] The identity of the $x_{L(max)}$ coordinate in both cases proves indirectly the complex stoichiometry.[90,93] The shift of the $x_{L(max)}$ to the left is joined with a complicated expression for the stoichiometric coefficient n of the $ML_n$ complex which becomes simple if the competing complex is MX and does not absorb in the applied wavelength interval:

$$n = \frac{x_{L(max)}}{1 - x_{L(max)}} + \frac{n[M]c_X}{c_0(1 - x_{max})([M] + \beta'_{MX})^2} \tag{29}$$

$$x_{L(max)} = \frac{1 - [M]^2 c_X/([M] + \beta'_{MX})c_0}{1 - 1/n} \tag{30}$$

For $[M] \gg \beta'_{MX}$, Equation 30 may be further simplified to

$$x_{L(max)} = \frac{1 - c_X/c_0}{1 + 1/n} \tag{31}$$

which enables a simple comparison of the theoretical $x_{L(max)}$ with its experimental value (see Figure 7). The disagreement of both values signals the presence of a mixed (ternary) complex according to the following equilibrium:

$$ML_n + pX \rightleftarrows ML_{n-p}X_p + nL \tag{F}$$

In the case of formation of weak complexes only with the competing ligand, the $x_{L(max)}$ value on the Job curve for the leading complex is not affected by the nonabsorbing competing ligand.[62] On the other hand, the presence of such a competing ligand may prevent the hydrolysis of the free metal ion into oligonuclear analyte complexes. The formation of oligonuclear analyte complexes leads to kinetic hindrances and causes unexpected deformations on the Job curve for the examined complex.[11,77,80,117]

The calculated normalized Job curves[77] give a detailed view across relations among stoichiometry, conditional stability constants of leading and competing complexes, and the concentration of competing ligand (see Figures 6 and 7).

### 2. *Competing Metal Ion Hydrolysis*

No shift of the maximum of the Job curves for analyte complexes ML, $ML_2$, etc. with various stoichiometries was observed when mononuclear hydrolytic complexes of considerable stability were formed simultaneously in solution. Nevertheless, the concentration of the leading analyte complex can be considerably suppressed. Considerable shifts of the expected coordinates of the Job curve maximum to the right, i.e., to larger $x_L$ values, however, take place when oligomeric hydrolysis occurs to $M_m(OH)_m$ or $M_m(OH)_n$. Additional curve deformations are also observed. Such competition can result in completely wrong results for the stoichiometry of the examined complex.[77,90] Time-dependent concavely curved deformations in the increasing part of the Job curve ($x_L < x_{L(max)}$) were often observed[117].

### 3. *Protonation and Deprotonation*

The $x_{L(max)}$ and the total shape of the Job curve is not influenced if protons are deliberated quantitatively during complexation, i.e., the metal ion complex being formed is a strong

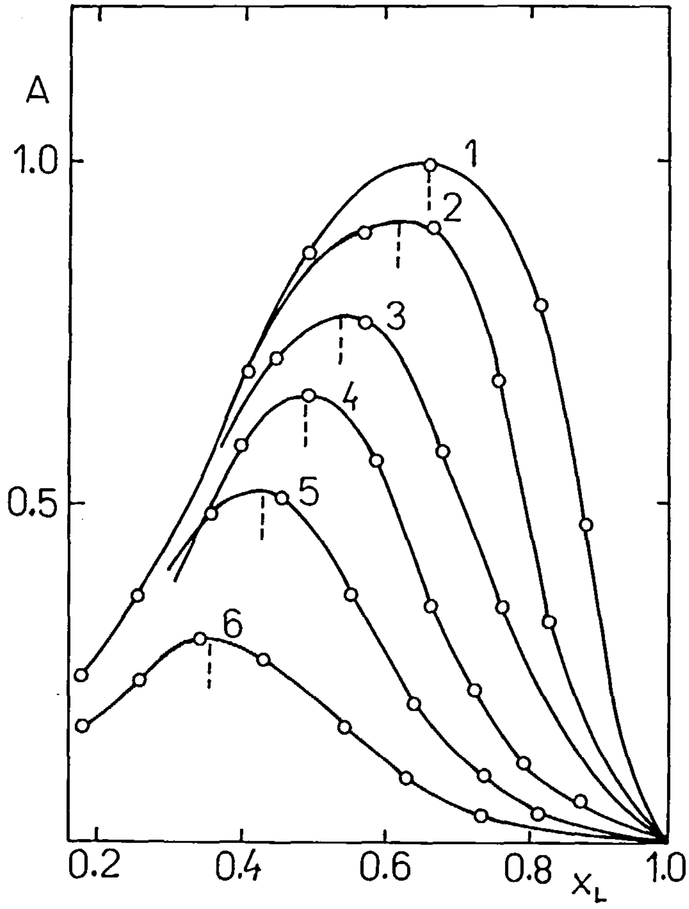

FIGURE 7. Competition between the $TiL_2$ complex of 1,8-dihydroxy naphthalene-3,6-disulfonic acid with EDTA ($H_2Y^{2-}$) dependent on EDTA concentration; a colorless TiY complex formed during the competition. Curve 1, without EDTA; curve 2, $2 \times 10^{-5}$ mol/ℓ EDTA; curve 3, $6 \times 10^{-5}$ mol/ℓ EDTA; curve 4, $10^{-4}$ mol/ℓ EDTA; curve 5, $1.4 \times 10^{-4}$ mol/ℓ EDTA. The experimental abscissa $x_L$ agrees with that calculated according to Equation 31.

acid.[64] This is usually not the case, and the pH must be carefully maintained constant during the whole series of solutions; otherwise a wrong shape of the curve and position of $x_{max}$ results. Since buffers must be used with care, often being competitive in the complex formation, pH is adjusted with strong diluted acid, ammonia, or alkaline hydroxide, or short pH plots are prepared for solutions of each component ratio in the solution series. The actual Job plots result then by vertical parallels belonging to various particular pHs.

The simultaneous formation of normal and protonated complexes of different molar absorptivity also does not interfere as long as a constant pH is maintained. The dissociation of the complexing ligand may also have no influence on the maximum of the Job curve if its sole form exists in solution during the examined pH interval.

The influence of various protonation and deprotonation equilibria on the position of the Job curve maximum of the examined complex has been discussed and summarized.[70]

In summary, the effective variant of the method of continuous variations in equimolar solutions is still a rapid and important method of testing the stoichiometry of complexes in solution under various limiting conditions, but the method is considerably less suitable for the evaluation of conditional stability or equilibrium constants.

## IV. GRAPHICAL LINEARIZATION PROCEDURES OF ABSORBANCE VS. PH AND ABSORBANCE VS. REACTANT CONCENTRATION PLOTS

Various graphical treatments of absorbance plots based on linearization and exploiting mathematical transformations of particular equilibrium constants have been developed and described in the literature, but only some of them have been used frequently in the spectrophotometric investigation of complexes. A vs. pH, A vs. $c_M$, or A vs. $c_L$ plots were interpreted under broad experimental conditions from which solutions with metal ion and reagent excess are especially of interest. Such graphical treatments are usually effective only when one complex equilibrium prevails in the particular complex formation interval.

Comparing curves for various $c_L$, $c_M$, or pH, the shape of the curve may suitably indicate a particular complex equilibrium in solution, e.g., the A vs. pH plot indicates the number of liberated protons, and the curve A vs. $c_L$ indicates the number of coordinated reagent species. The curves become steeper if more protons are liberated or more ligand species coordinated. Curves may be modeled or simulated by a computer according to a suitable program (e.g., the HALTAFALL SPEFO GRAPH program[15]) and compared with the normalized experimental curves. Normalized terms

$$y = A/A_0 = A/\epsilon_k c_M \tag{32}$$

for a complex formation from the components or

$$y = \frac{A - \epsilon_1 c_M}{\epsilon_2 c_M - \epsilon_1 c_M} = \frac{A - A_{01}}{A_{02} - A_{01}} \tag{33}$$

for a transition between two complexes are plotted against pH or $c_L$ concentration in excess at constant pH and compared with plots calculated for various tested equilibria. The formation of mononuclear and dimeric species may be distinguished in this way (see Figures 8D and 9).

The family of A vs. pH plots (Figure 8A) indicates two separated equilibria corresponding with the successive formation of two complexes. The shift of the plots to more acidic interval with the increasing concentration $c_L$ ($c_{L1} < c_{L2} < c_{L3}$) proves the coordination of the reagent and liberation of protons in both steps. By analyzing successively singular pH plots in separated formation intervals, the number of liberated protons is determined. The ratio of the number of protons liberated and the number of ligand species coordinated may be established in various constant absorbance levels during the whole formation interval (interpretation of corresponding solutions). In Figure 8B, a mononuclear complex by reagent coordination in the first step is formed, but curves are not influenced by changes in the reagent concentration since the reagent does not take part in complexation in the second step. Concerning Figure 8C, the coordination of reagent takes place in the first step, as in the case for A and B, whereas an inverse shift of curves is observed in the second formation interval since a reagent species is split and a hydroxocomplex formed.

The interpretation of curves follows *per partes* for successive formation intervals. Tests for various complex equilibria over the absorbance plots are carried out by (1) a direct graphical analysis using suitable slope-intercept transformations of the equilibrium or conditional stability constant corresponding to the assumed equilibrium being tested, or (2) by logarithmic graphical analysis using similar transformations, which, however, require the previous evaluation of the molar absorptivity, e.g., from the slope-intercept nonlogarithmic plots or the limiting plateaus of such plots. For the sake of simplicity, two kinds of complex equilibria are assumed: (1) the formation of a complex from the components and (2) the transition between two complexes, neglecting the free concentration of the minor component.

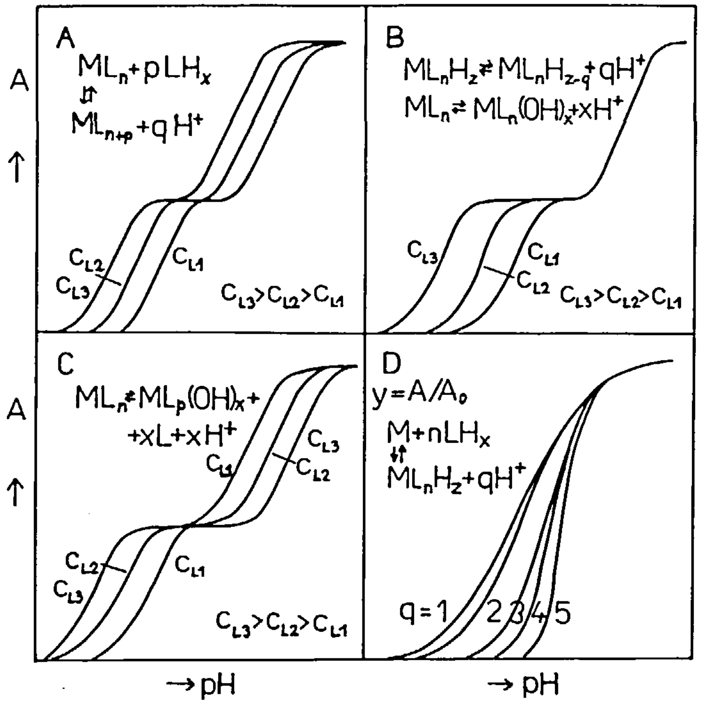

FIGURE 8. Various forms of absorbance vs. pH plots. (A to C) Various constant excess of the complexing agent; (D) shapes of normalized plots with respect to the number of protons deliberated during complex formation. The curves indicate different complex equilibria in solution.

In addition, the concentration of the reagent bound into the complex is neglected for solutions with an excess of reagent, as is the concentration of complexed metal ion with respect to the metal ion excess in solution. On the other hand, successive approximation of the free concentration of components besides the complexes in solution may be carried out in the case of equimolar solutions.

## A. Studies Using Slope-Intercept Transformations of Equilibrium Constants

Typical slope-intercept transformations for solutions with an excess of metal ion or reagent related to plots

$$c_M/A \quad \text{vs.} \quad [H]^z,\ c_L^n \qquad A \text{ vs. } [H]^z,\ c_L^n$$

$$c_M/A \quad \text{vs.} \quad [H]^z,\ c_M^m \qquad A \text{ vs. } [H]^z,\ c_M^m$$

are summarized in Table 3. The straight line course of the graphical plot of the particular slope-intercept transformation proves the tested or assumed equilibrium and gives evidence of the stoichiometric coefficients of the components in the complex formed or the number of liberated protons during complexation (see Figure 10). For simultaneous side reactions such as reagent dissociation and protonation or metal ion hydrolysis, correction terms were introduced into the equations (see Table 3). The interpretation of absorbance curves becomes, however, rather complicated for equimolar or nearly equimolar concentrations of reacting components because of complicated forms of transformations and necessary successive approximation procedures.

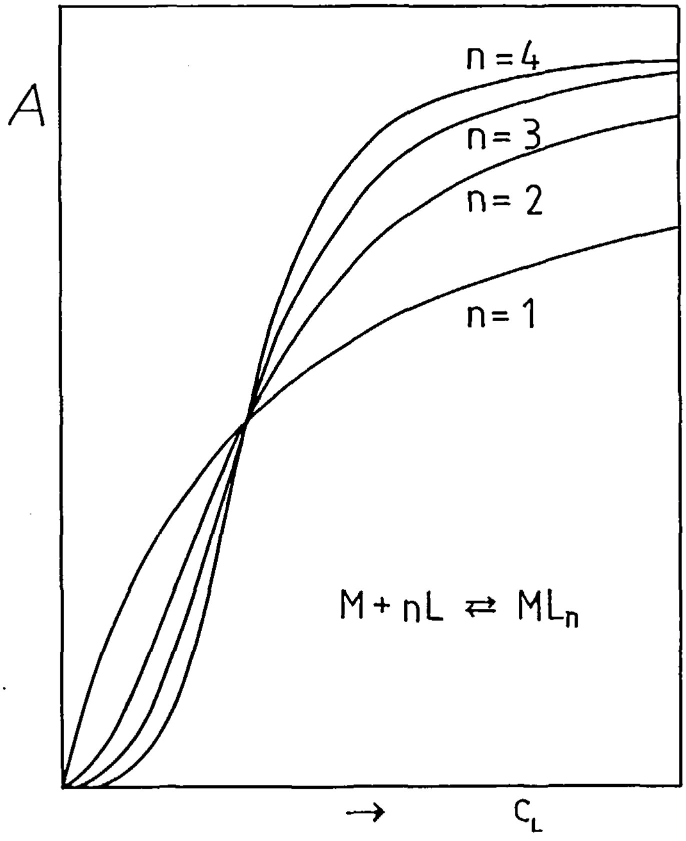

FIGURE 9. Comparison of absorbance vs. reagent concentration plots for various stoichiometries of the $ML_n$ complex being formed.

The actual molar absorptivity of the particular complex results from graphical limits of $c_i/A$ or A transformation. Thus, correct values may also be obtained from the curved lines or curved transformation plots with incorrect exponential integers in expressions. A successive approximation of limiting values of molar absorptivity is provided for nearly equimolar concentrations of components. For mutual transitions of two complexes, the A transformation form is more suitable. The knowledge of the molar absorptivity of one complex is obligatory in such a case, but satisfying results are also obtained starting the interpretation from at least three values of absorbance near the true limiting plateau. Plateaus on absorbance plots which often correspond to the quantitative complex formation are very helpful for comparison or serve as first estimates of $A_{01}$ in the transformation. In horizontal parts (plateaus) of the absorbance plot, the particular complex need not, however, be formed quantitatively even in the presence of component excesses. This must be considered if the molar absorptivity is calculated from such plateaus. Such an absorptivity value should be checked with that calculated or extrapolated from the limit of above slope-intercept transformations. The equilibrium or conditional stability constant can be calculated from the slope of linear slope-intercept transformation, but the joint logarithmic transformation is more suitable. The value of the molar absorptivity found from the limiting intercept of the nonlogarithmic transformation is introduced into the corresponding logarithmic expression, and more precise values of stoichiometric coefficients result from the slope and the equilibrium constant calculated from the points.

Such slope-intercept transformations may also be evaluated numerically by using least-squares procedures with simple programs in ALGOL or FORTRAN and desk calculators.

## Table 3

## SOME SELECTED SLOPE-INTERCEPT TRANSFORMATIONS OF THE EQUILIBRIUM CONSTANTS FOR EVALUATION OF COMPLEX EQUILIBRIA

**Solutions with an Excess of Metal Ion ($c_M > c_L$)**

Equilibrium:

$\mathrm{M} + \mathrm{LH}_x \rightleftarrows \mathrm{MLH}_z + q\mathrm{H}^+$ $\quad {}^*\beta_1$
$\mathrm{LH}_x \rightleftarrows \mathrm{LH}_{x-1} + \mathrm{H}^+$ $\quad K_a$
$\mathrm{M} + j\mathrm{H}_2\mathrm{O} \rightleftarrows \mathrm{M(OH)}_j + j\mathrm{H}^+$ $\quad {}^*\beta_j$ (UE5)

Transformations:

$$c_L/A = 1/\epsilon_k + \frac{AZ_HZ_{OH}[H]^q}{{}^*\beta_1\epsilon_k c_M A}$$
$$A = \epsilon_k c_L - AZ_HZ_{OH}[H]^q/{}^*\beta_1 c_M$$
$$A = \epsilon_k[\mathrm{MLH}_z] + \bar{\epsilon}_L c_L$$
$$Z_{OH} = 1 + \Sigma{}^*\beta_j/[H]^j$$
$$Z_H = 1 + K_a/[H]$$
$$\log\frac{AZ_HZ_{OH}}{\epsilon_k c_L - A} = q\,\mathrm{pH} + \log c_M + \log{}^*\beta_1 \qquad \text{(UE6)}$$

Equilibrium:

$m\mathrm{M} + n\mathrm{LH}_x \rightleftarrows \mathrm{M}_m\mathrm{L}_n\mathrm{H}_z + q\mathrm{H}^+$
$\mathrm{LH}_x \rightleftarrows \mathrm{LH}_{x-1} + \mathrm{H}^+$ $\quad K_a$ (UE7)

Absorbance:

$\epsilon_k[\mathrm{M}_m\mathrm{L}_n\mathrm{H}_z] + \epsilon_{\mathrm{LH}_x}[\mathrm{LH}_x] + \epsilon_{\mathrm{LH}_{x-1}}[\mathrm{LH}_{x-1}] + \epsilon_M c_M$ (UE8)

Transformations:

$$c_L/(A - \epsilon_M c_M) = n/\epsilon_k + \frac{[Z(A - \epsilon_M c_M) - \bar{\epsilon}_L c_L](n\bar{\epsilon}_L - \epsilon_k Z)^{n-1}}{(nA - \epsilon_k c_L - n\epsilon_k c_M)^{n-1}(A - \epsilon_M c_M)}\,\frac{[H]^q}{{}^*\beta\epsilon_k c_M^m}$$
$$A - \epsilon_M c_M = \epsilon_k c_L/n - \frac{Z(A - \epsilon_M c_M) - \bar{\epsilon}_L c_L(n\bar{\epsilon}_L - \epsilon_k Z)^{n-1}[H]^q}{(nA - \epsilon_k c_L - n\epsilon_M c_M)^{n-1}nc_M^m\,{}^*\beta}$$
$$\log\frac{[(Z(A - \epsilon_M c_M) - \bar{\epsilon}_L c_L)(Z\epsilon_k - n\bar{\epsilon}_L)^{n-1}}{[\epsilon_k c_L - n(A - \epsilon_M c_M)]^n} = q\,\mathrm{pH} + m\log c_M + \log{}^*\beta$$
$$\bar{\epsilon}_L = \epsilon_{\mathrm{LH}_x} + \epsilon_{\mathrm{LH}_{x-1}}([H]/K_a)^{-1}$$
$$Z = 1 + K_a/[H] \qquad \text{(UE9)}$$

**Solutions with an Excess of Ligand ($c_L > c_M$)**

Equilibrium:

$\mathrm{M} + n\mathrm{LH}_x \rightleftarrows \mathrm{ML}_n\mathrm{H}_z + q\mathrm{H}^+$ $\quad {}^*\beta$
$\mathrm{LH}_x \rightleftarrows \mathrm{LH}_{x-1} + \mathrm{H}^+$ $\quad K_a$ (UE10)

Absorbance:

$A = \epsilon_k[\mathrm{ML}_n\mathrm{H}_z]$

## Table 3 (continued)
## SOME SELECTED SLOPE-INTERCEPT TRANSFORMATIONS OF THE EQUILIBRIUM CONSTANTS FOR EVALUATION OF COMPLEX EQUILIBRIA

**Solutions with an Excess of Ligand ($c_L > c_M$)**

Transformations:

$$c_M/A = 1/\epsilon_k + \frac{Z^n[H]^q}{\epsilon_k c_L^n {}^*\beta}$$
$$A = \epsilon_k c_M - AZ^n[H]^q/c_L^n {}^*\beta$$
$$\log \frac{AZ^n}{\epsilon_k c_M - A} = q\ pH + n \log c_L + \log {}^*\beta$$
$$Z = 1 + K_a/[H]$$

Equilibrium:

$$ML_n + pLH_x \rightleftarrows ML_{n+p}H_z + qH^+$$
$$LH_x \rightleftarrows LH_{x-1} + H^+ \qquad K_p$$

Absorbance:

$$A = \epsilon_1[ML_n] + \epsilon_2[ML_{n+p}H_z]$$

Transformations:

$$A = \epsilon_2 c_M - \frac{(A - \epsilon_1 c_M)Z[H]^q}{K_p c_L^p}$$
$$A = \epsilon_1 c_M + (\epsilon_2 c_M - A)K_p c_L^p/Z[H]^q$$
$$\log \frac{A - \epsilon_1 c_M}{\epsilon_2 c_M - A} Z = q\ pH + \log c_L + \log K_p$$
$$Z = 1 + K_a/[H]$$

Equilibrium:

$$vM_aL_bH_cX_g + s\ LH_x \rightleftarrows dM_m\ L_nH_zX_l + fXH_j + qH$$
$$LH_x \rightleftarrows LH_{x-1} + H^+$$

Absorbance:

$$A = \epsilon_1[M_aL_bH_cX_g] + \epsilon_2[M_mL_nH_zX_l] + \epsilon_{LH_x}[LH_x] + \epsilon_{LH_{x-1}}[LH_{x-1}]$$

Transformations:

$$A - A_{0L} = \epsilon_2 c_M/m - \frac{[a(A - A_{0L}) - \epsilon_1 c_M]^d[H_jX]^f Z^s(a\epsilon_2 - m\epsilon_1)^{v-d}[H]^q}{[\epsilon_2 c_M - m(A - A_{0L})]^{v-1} c_L^s m^*\beta}$$
$$\log \frac{[\epsilon_1 c_M - a(A - \bar{\epsilon}_L c_L')]^d}{[m(A - \bar{\epsilon}_L c_L') - \epsilon_2 c_M]^v} = q\ pH + s \log c_L' - f[XH_j] - (v - d) \log(m\epsilon_1 - a\epsilon_2) + \log {}^*\beta$$
$$A_{01} = \epsilon_1 c_M/a; \quad A_{02} = \epsilon_2 c_M/m; \quad Z = 1 + K_a/[H]$$
$$A_{0L} = \epsilon_L c_L/Z; \quad c_L' = c_L/Z$$
$$\bar{\epsilon}_L = \epsilon_{LH_x} + \epsilon_{LH_{x-1}}(K_a/[H])$$

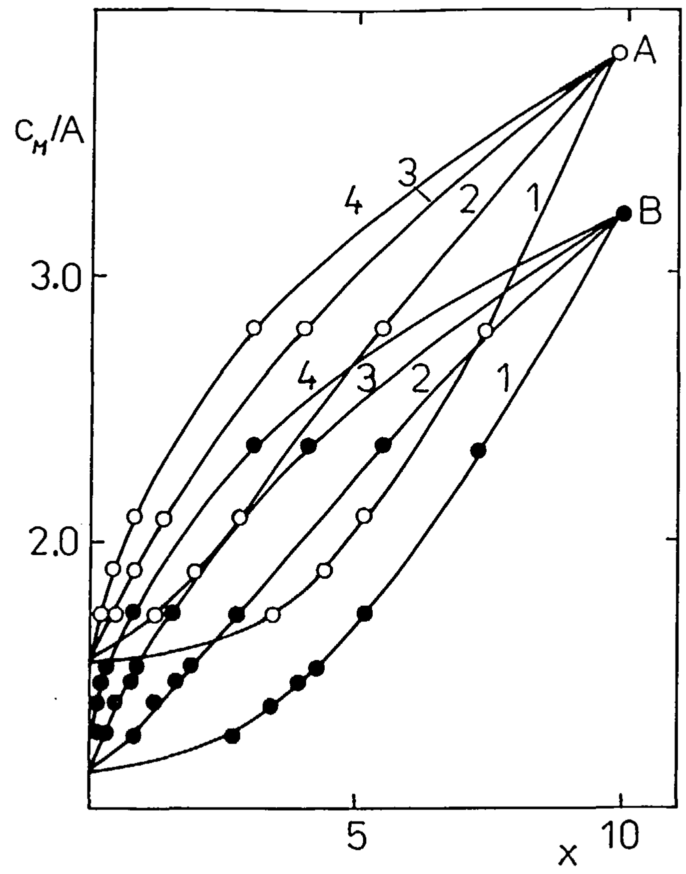

FIGURE 10. Shapes of $c_M/A$ slope-intercept transformations in the system Ti(IV)-1,2-dihydroxybenzene-3,5-disulfonic acid. $c_M/A = 1/\epsilon + [H]^q/\epsilon^*\beta c_L^n$; $c_M = 1.2 \times 10^{-4}$ mol/ℓ; $c_L = 1.5 \times 10^{-2}$ mol/ℓ. Curve 1, [H]; curve 2, $[H]^2$; curve 3, $[H]^3$; curve 4, $[H]^4$; straight-line course proved q = 2; (A) 460 nm; (B) 430 nm.

A variant of the least-squares program PRCEK[118,119] is suitable for the evaluation of normal and logarithmic slope-intercept transformations describing acid-base and complex equilibria with binary complexes, and LITRAFAL[120] is suitable for study of ternary complexes. Using the PRCEK program, the calculation of all necessary parameters of the complexation equilibrium is carried out in one run or by manifold approximation. The rough values of $A_{01}$ and $A_{02}$, or $\epsilon_1$ and $\epsilon_2$, respectively, if accessible, are introduced with assumed or tested exponential integers m, n, and q into particular equations. Assuming Equilibrium G,

$$M + nH_iL \rightleftarrows ML_nH_z + qH^{+*}\beta_n \tag{G}$$

the following equations are valid for calculation:

$$A = \epsilon_1 c_M + F_1{}^*\beta_n \tag{34}$$

$$A = \epsilon_2 c_M + F_2{}^*\beta_n \tag{35}$$

$$F_1 = (\epsilon_2 c_M - A)c_L^n/[H]^q \tag{36}$$

$$F_2 = (A - \epsilon_1 c_M)[H]^q/c_L^n \tag{37}$$

$$A_{calc} = \frac{c_M\{\epsilon_1[H]^q + \epsilon_2 c_L^n {}^*\beta_n\}}{[H]^q + c_L^n {}^*\beta_n} \tag{38}$$

The choice of best values of stoichiometric coefficients is carried out with respect to the correlation coefficient $r_{xy} > 0.95$ and the minimum value of

$$U = \sum^{np} \sum^{n\lambda} (A_{calc} - A_{exp})^2 \tag{39}$$

either for the direct or logarithmic treatment of absorbance plots. The computer repeats calculations and successively improves the values of molar absorption coefficients and of the equilibrium constant until $\Delta A_{01}$ or $\Delta A_{02} \leq 0.002$, or $\Delta A_{01}/A_{01} \sim 0.1\%$, but it stops the calculation after six runs for wrongly assumed equilibrium. The best values of molar absorptivities and the equilibrium constant are used for calculation of $A_{calc}$ and to check the U condition. The optimum value of $\epsilon_i$ is introduced by the computer in the logarithmic transformation log F vs. pH, log $c_M$, or log $c_L$, and stoichiometric coefficients and $^*\beta_n$ are newly calculated. Some 10 to 20% of the points near the limiting parts of the formation interval are omitted during the interpretation.[121]

Correct values for stoichiometric coefficients and equilibrium constants only result from logarithmic slope-intercept transformations if true values of molar absorptivities are inserted into equations and correction terms for side reactions are respected. The equilibrium or conditional stability constant of the complex studied may simply be calculated from (1) the pH or free ligand or metal ion concentration in solution, for which the left side of the particular logarithmic transformation tends to zero value; or (2) any point of the logarithmic straight line. Some of the logarithmic transformations commonly used in our previous work are shown in Table 3 and published work.[9,11,14,122] Various linear slope-intercept transformations of equilibrium and conditional stability constants of the previous type and their logarithmic forms were proved during the interpretation of A vs. pH, A vs. $c_M$, or A vs. $c_L$ plots in studies of complex equilibria of different organic analytical reagents.[11,118,121,123-135] Complicated complex equilibria could be revealed in solution in this way, even during some apparently simple analytical reactions (see Figure 11).

These procedures are most effective for studies of complex equilibria with medium stable complexes if the complex formation intervals may be sufficiently separated by suitable working conditions. Absorbance plots for very stable complexes can be interpreted only with difficulty since the absorbance interval to be interpreted is very short. For such a case, the simple mole ratio method[136-138] or some modified logarithmic interpretation[127] may be used with more success. If very weak complexes are formed in solution, large excesses of concentration of the components are necessary, which seriously increase the absorbance blank, and the concentration of reagent is limited by its actual solubility. The interpretation becomes rather complicated if more complex equilibria overlap and significantly participate on the actual absorbance or if complicated side reactions occur.

Analogous linear slope-intercept transformations also gave satisfying results when applied in the systems with ternary complexes considering or not considering substitution equilibria during their formation from corresponding binary species. The dissociation of the related binary species is neglected with advantage, as is the absorptivity of the free components.[120,139-141] Similarly, equations for the logarithmic interpretation of absorbance plots related to the formation of ternary species from binary species considering substitution equilibria were derived.[142]

The stoichiometry of the complex, the molar absorptivity, and the conditional stability constant for complexes of limited stability may be determined by a procedure resembling

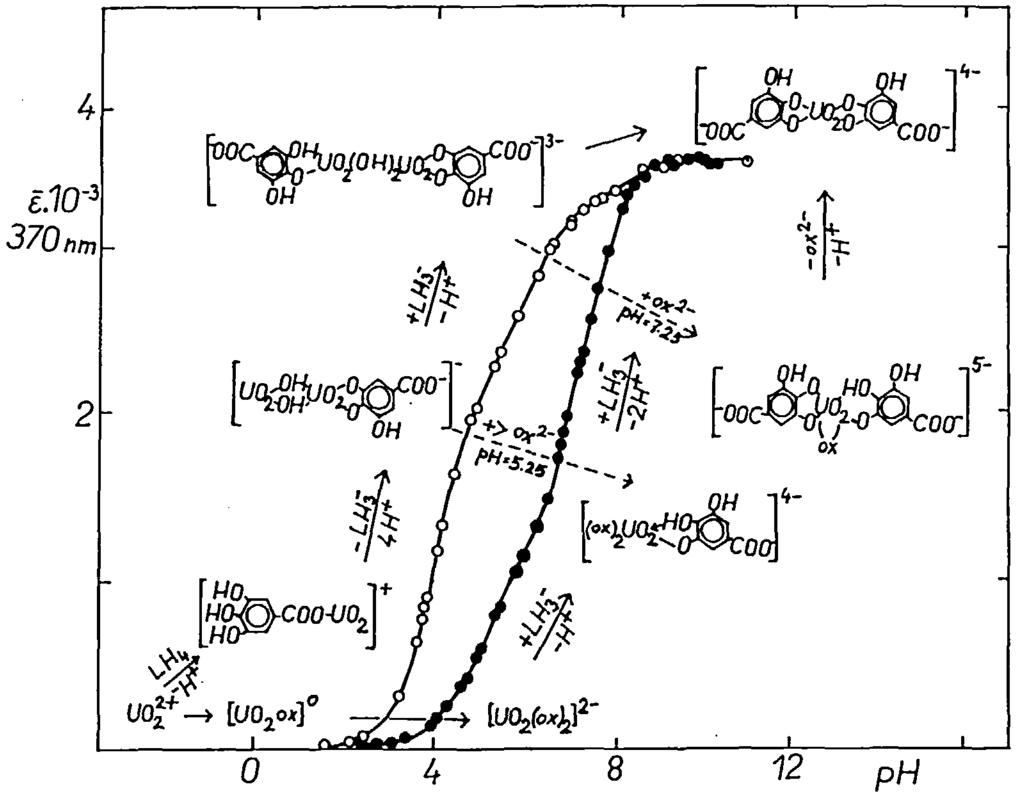

FIGURE 11. Molar absorptivity vs. pH plots in the system $UO_2^{2+}$-gallic acid (○) and oxalic acid and gallic acid (●) dependent on pHi $c_M = 3.8 \times 10^{-4}$ mol/ℓ; $c_{gal} = 1.9 \times 10^{-2}$ mol/ℓ; $c_{ox} = 1.9 \times 10^{-2}$ mol/ℓ; 370 nm; an example of complicated complex equilibria examined by pure graphical analysis (Job curves, direct and logarithmed slope-intercept transformations).

the exploitation of spectrophotometric titration plots.[143,144] This is useful for dye complexes, where a large reagent excess is not applicable. A straight-line logarithmic plot (formation of a ML complex)

$$\log \frac{1}{(c_M/c_L)\Delta A_0 - \Delta A} = -\log \frac{\Delta A}{\Delta A_0 - \Delta A} + \log \beta'_1 - \log \Delta\epsilon_{ML} \qquad (40)$$

referring to solutions with various constant $c_L$ and variable $c_M$, proves the stoichiometry of complex. For a general complex $M_mL_n$,

$$\Delta A = A - \epsilon_L c_L, \quad \Delta A_0 = \Delta\epsilon_{M_mL_n} c_L/n, \quad \text{and} \quad \Delta\epsilon_{M_mL_n} = \epsilon_{M_mL_n} - n\epsilon_L$$

The difference molar absorptivity results successively from various apparent values of molar absorptivity which are expressed by tangents of A vs. $c_M$ plots for various $c_L$ constants:

$$\lim \left(\frac{d\Delta A}{dc_M}\right)_{c_M \to 0} = \Delta\epsilon'_{ML_n(app)} = \Delta\epsilon_{ML_n} \frac{c_L^n}{c_L^n + 1/\beta'} \qquad (41)$$

or evaluated for solutions with large $c_M$ excess. It is related to the true difference molar absorptivity and conditional stability constant by the following plot:

$$c_L^n = \Delta\epsilon_{ML_n} c_L^n / \Delta\epsilon_{ML_n(app)} - 1/\beta' \qquad (42)$$

where $\Delta\epsilon_{MLn(app)} = \Delta A/c_M$ for solutions with $c_M < nc_L$ or $n\Delta A/c_L$ for solutions with $c_M > n\ c_L$. Similarly, the calibration plots, like the spectrophotometric titration curves, are influenced by the concentration of reagent, particularly the slope of such plots in the origin when considerably dissociated complexes are formed.[145] Various simple linear slope-intercept transformations mentioned earlier in the literature may be valid for studies of simple complex equilibria.[146-163,206]

Similarly, the logarithmic interpretation of absorbance plots was introduced early as the method of limit logarithm for studies of weak complexes,[164,165] or simplified logarithmic transformations were frequently used for medium stable complexes, often omitting serious limitations of such application.[5,166-171]

Some different forms of logarithmic slope-intercept expression were described with respect to more acid-base species simultaneously formed in solution or complicated hydrolytic equilibria of the metal ion:

$$\log B = nq\ \mathrm{pH} - \log C \tag{43}$$

where q is the number of liberated protons and C is a constant. The stoichiometric coefficient n of the complex $ML_n$, which is also implicitly included in term B, must be successively tested assuming various hydrolytic or acid-base species until linear plots of log B vs. pH are obtained. Such transformations are less suitable for the evaluation of equilibrium constants.[172-176]

Stable complexes with very short formation absorbance plots require equimolar or nearly equimolar solutions, or solutions with a very small excess of reagent for study. In addition, such plots have well-developed limiting horizontal plateaus, where

$$A_0 = \epsilon_k c_M \tag{44}$$

The concentration of the studied complex is then expressed by

$$c = Ac_M/A_{0(max)} \tag{45}$$

where $c_M$ is the total concentration of the metal ion. The number of liberated protons may be easily evaluated from the slope of the linear course of such plots if the correct stoichiometry of the complex species is previously known or assumed.[127]

A simple combination of some kind of slope-intercept A transformation with the related logarithmic expressions was used earlier to study systems with three absorbing complexes, including ternary species in solutions with various concentrations and various wavelengths.[20] No information is given by this procedure for the proton balance in equilibria studied.

## B. The Straight-Line Method

The straight-line method[177] is another variant of the graphical analysis of absorbance plots with special reference to absorbance vs. metal ion or reagent concentration plots at a constant pH. The corresponding equations represent a special case of slope-intercept transformation whose straight-line course for inserted particular small integers as exponents m and n in the equations also gives evidence about the number of ligands being coordinated to the metal ion or the number of metal ions in the complex formed, the molar absorptivity of the complex, and sometimes its conditional stability constant. For complicated equilibria or the formation of complicated complexes, the equations become rather composed. Equations for two general equilibria are mentioned here as examples in solution with ligand excess ($c_L > c_M$):

$$mM + nLH_x \rightleftarrows M_mL_nH_z + qH^+ \quad {}^*\beta_{mn} \tag{H}$$

$$LH_x \rightleftarrows LH_{x-1} + H^+ \quad K_a \tag{I}$$

$$c_M = [M] + m[M_mL_nH_z] \tag{46}$$

$$c_L = Z[LH_x] + n[M_mL_nH_z] \tag{47}$$

$$Z = 1 + K_{ax}/[H] \tag{48}$$

$$A = \bar{\epsilon}_L[LH_x] + \epsilon_M[M] + \epsilon_k[M_mL_nH_z] \tag{49}$$

$$\bar{\epsilon}_L = \epsilon_{LH_x} + \epsilon_{LH_{x-1}}K_{ax}/[H] \tag{50}$$

$$\begin{aligned} 1/[LH_x]^n \cong 1/c_L &= \frac{[M]^{m*}\beta_{mn}}{[M_mL_nH_z][H]^q} = \frac{X}{Y}(c_M - mY/X)\frac{*\beta_{mn}}{[H]^q} \\ &= \text{constant } c_M^m X\left[\frac{1}{Y} + \binom{m}{2}\left(\frac{m}{Xc_M}\right)^2 Y - \binom{m}{3}\left(\frac{m}{Xc_M}\right)^3 Y^2 + \ldots \right. \\ &\quad \left. + (-1)^m\left(\frac{m}{Xc_M}\right)^m Y^{m-1} - \frac{m^2c_M^{m-1}{*\beta_{mn}}}{[H]^q}\right] \end{aligned} \tag{51}$$

$$Y = Z(A - \epsilon_Mc_M) - \bar{\epsilon}_Lc_L \tag{52}$$

$$X = Z(\epsilon_k - \epsilon_M) - \bar{\epsilon}_Ln \tag{53}$$

If a mononuclear complex is formed, the equations become simpler:

$$M + nLH_x \rightleftarrows ML_nH_z + qH^+ \tag{J}$$

$$\begin{aligned} 1/[LH_x]^n = 1/c_L^n &= \frac{X}{Y}\left(c_M - \frac{Y}{X}\right)\frac{*\beta_{1n}}{[H]^q} \\ &= \frac{*\beta_{1n}c_MX}{[H]^q}\frac{1}{Y} - \frac{*\beta_{1n}}{[H]^q} \end{aligned} \tag{54}$$

The developed binomial absorbance terms appear in the particular transformations only for cases in which oligonuclear complexes are formed in solutions with a ligand excess or when some higher complex with $n > 1$ is formed in solutions with an excess of metal cations. However, the terms with $m \geqslant 2$ or $n \geqslant 2$ that tend to zero have a negligible influence on the graphical plots and may be neglected, so that simplified expressions are usually plotted, e.g., $1/[LH_x]^n$ vs. $1/Y$ or $1/c_m^m$ vs. $1/Y$, where Y is a more or less composed absorbance term. The straight-line course of such plots for true m and n is practically not influenced. This was also demonstrated earlier.[178]

The interpretation of such straight-line transformations is suitable for solutions with one reacting component in excess and the other one in variable concentrations maintaining pH constant. Various forms of corresponding transformations may be listed:

(1) for solution with reagent excess:

$$1/[LH_x]^n \quad \text{vs.} \quad 1/Y \tag{55}$$

related to A vs. $c_L$ plots with constant metal ion concentration or

$$1/c_M^m \quad \text{vs.} \quad 1/Y \tag{56}$$

related to A vs. $c_M$ plots for solutions with variable minor $c_M$ concentrations, or (2) for solutions with excess of metal cations:

$$1/[M]^m \quad \text{vs.} \quad 1/Y \tag{57}$$

related to A vs. $c_M$ plot for solutions with constant ligand concentration, or (3)

$$1/c_L^n \quad \text{vs.} \quad 1/Y \tag{58}$$

related to A vs. $c_L$ plots for solutions with variable small concentration of $c_L$. As usual, small integers are successively inserted for m or n into the particular expressions and the straight-line course tested.

A great invention of the author[178] was to replace the concentration of components with volumes of stock solutions of components in the particular transformations and plot volume terms against the absorbance terms maintaining the total solution volume constant. This is valid since

$$c_i = v_i(c_i)_0/V = \text{constant } v_i \tag{59}$$

and enables the use of not fully defined reagent samples, which is especially suitable for dyes and other organic reagents.

Correction terms with respect to simultaneous protonation or dissociation of the reagent or hydrolysis of the metal ion may also be inserted and are of interest when the conditional stability constant of the complex is calculated. Expressions for the formation of ternary species under different conditions were also derived.[179] The application and development of the straight-line method in aqueous solution[178,180,181] and during solvent extraction of complex species[182,183] are discussed in the literature; a more comprehensive discussion has also been published.[11] Further improvement of this method, which is especially suitable for weak complexes of some nonmetals, was realized by respecting the limiting absorbance for solutions with a large excess of one component.[180,184] The reagent bound into the complex is not neglected in this case. To determine the stoichiometry of a complex $M_mL_n$, the value of m/n is evaluated from a plot of $1/[LH_x]^{n/m}$ vs. 1/A for a solution with a variable concentration of the component M in excess and a constant low concentration of L; the value of n for another series of solutions with variable $c_L$ and constant excess of $c_M$ is evaluated according to a plot of $1/[LH_x]^n$ vs. 1/A. The formation of the same complex with particular m/n stoichiometry is ensured.

In summary, the straight-line procedure is sometimes suitable for the evaluation of complex stoichiometry, even to distinguish between monomeric and dimeric species, but it is not very effective for the evaluation of conditional stability constants of complexes. The study of weak or medium stable complexes is preferred in applying this method.

## C. Evaluation of Corresponding Solutions

Corresponding solutions are solutions containing various concentrations of reacting components at the same pH or with the constant concentration of one component and pH and the variable concentration of the other reacting component which shows the same absorbance value. Such absorbance plots may be evaluated in a graphical or numerical way. A simple variant of such a procedure is based on pairs of absorbance plots vs. variable concentrations of reagent in small excess for various constant concentrations of metal ions and constant pH or vice versa. The Bjerrum average coordination number $\bar{n}$ and the formation function simply follow from the expressions

$$\bar{n} = \frac{c'_L - c''_L}{c'_M - c''_M} \tag{60}$$

$$[L] = (c'_M c''_L - c''_M c'_L)/(c'_M - c''_M) \tag{61}$$

where $c'_M$, $c''_M$, $c'_L$, and $c''_L$ are the corresponding concentrations of the metal ion and the ligand. The quasilinear parts of such plots have to be evaluated.[185-188] This simple treatment has often given erroneous or ambiguous results and has been modified.[187] An attempt to study nonabsorbing complexes in competition with a highly colored indicator complex of the same metal ion has also been described.[9,189,190]

For families of stepwise-formed complexes, the principle of correspondence has been successfully joined with the concept of average molar absorptivity, defined as[191,192]

$$\bar{\epsilon}_M = A/c_M = \frac{\epsilon_0 + \epsilon_1\beta'_1[L] + \epsilon_2\beta'_2[L]^2 + \ldots + \epsilon_n\beta'_n[L]^n}{1 + \beta'_1[L] + \beta'_2[L]^2 + \ldots + \beta'_n[L]^n} \tag{62}$$

and

$$\Delta\bar{\epsilon}_M = \frac{\Delta\epsilon_1\beta'_1[L] + \Delta\epsilon_2\beta'_2[L]^2 + \ldots + \Delta\epsilon_n\beta'_n[L]^n}{1 + \beta'_1[L] + \beta'_2[L]^2 + \ldots + \beta'_n[L]^n} \tag{63}$$

where $\Delta\bar{\epsilon}_M = \bar{\epsilon}_M - \epsilon_0$, $\Delta\epsilon_1 = \epsilon_1 - \epsilon_0$, $\Delta\epsilon_n = \epsilon_n - \epsilon_0$, and the formation of mononuclear complexes only is assumed. The $\bar{\epsilon}_M$ vs. $c_L$ plots were interpreted for various constant minor $c_M$ or pH. A simple graphical treatment results for the system with M, ML, and $ML_2$ only for which the molar absorptivities are evaluated from the intercepts and the constants $\beta'_i$ from the slopes of derived straight-line functions.[193] In general, equations derived from Equation 62 correspond without approximation to $2n + 2$ independent unknown parameters, and the numerical treatment may give confusing results if more than three complexes are formed simultaneously.[7,194] In the past, this basic equation was successively modified to auxiliary functions whose graphical limitations to zero with respect to [L] or 1/[L] gave successively $\epsilon_i - \epsilon_0$ and $\beta'_i$ values.[192,195-197] The evaluation of particular parameters from two sets of auxiliary-derived functions from Equation 63 is simple and successful for two complexes of limited stability.[192] Such a procedure was extended and modified for more stepwise-formed mononuclear complexes maintaining the advantageous graphical extrapolation of parameters.[197] Such procedures became complicated for sets of oligonuclear complexes in solution[198] and lost their significance after introduction of computers for searches for equilibria in solution. The number and stoichiometry of complexes being formed simultaneously are indirectly proved only by the above procedures-comparing the reality and precision of resulting parameters. The $\bar{\epsilon}_M$ vs. pH plots for various $c_M$ may also be interpreted in the above way, which may be useful for studies of hydrolytic equilibria.[199]

Another useful exploitation of the principle of solution correspondence is for separated and single complex equilibria from families of absorbance plots against pH or the particular reacting component. In this case, solutions with the same concentration of complexes or absorbance terms but with different pHs or concentration of variable components result for parallels along the abscissa during the whole formation interval of the complex. At the particular absorbance term, the following expressions have constant values:

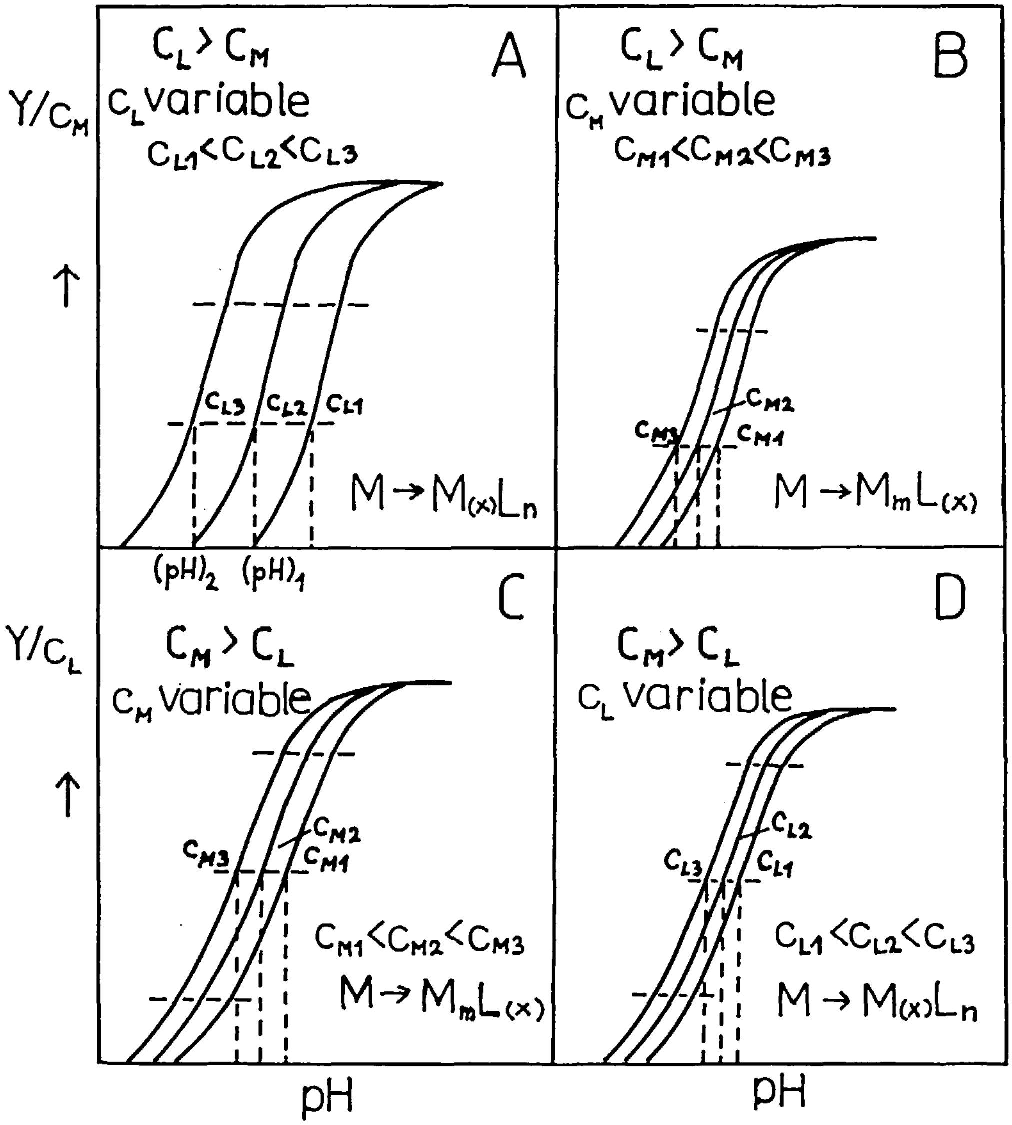

FIGURE 12. Exploitation of the principle of correspondence for sets of absorbance vs. pH plots under various conditions (with one equilibrium always prevailing). Logarithmic evaluation of n/q (A) or m/q (C), respectively, for parallels against the abscissa pH. If plots for various $c_M$ overlap (B), no oligonuclear complex is formed; if plots for various $c_L$ overlap (D), no higher complex with n > 1 is formed.

$$[M_mL_nH_z]/[M]^m; \quad [M_mL_nH_z]/[H_xL]^n; \quad [M_mL_nH_z]/[M_aL_bH_c]$$

or simply from the absorbance plot:

$$n(A - A_M)/c_M \quad \text{or} \quad n(A - A_L)/c_L \tag{64}$$

for solutions with metal ion or ligand excess. If the reagent or metal ion in minor variable concentration also takes part in competing acid-base equilibria or the reacting components absorb, logarithmic plots are necessary for the evaluation of the corresponding condition. Various parts of the absorbance plots suitable for evaluation via corresponding solutions are indicated in Figure 12. The ratios of various stoichiometric indexes result from the slopes of very simple logarithmic plots related to corresponding solutions (see Table 4). A detailed discussion of this problem appears in the literature.[11]

## Table 4
## EVALUATION OF STOICHIOMETRIC COEFFICIENTS FROM CORRESPONDING SOLUTIONS

| **Absorbance or log absorbance plots** | **Conditions** | **Log plots valid for correspondence; complex $M_mL_n$ formed; q protons liberated** | |
|---|---|---|---|
| A vs. pH | Constant $c_L$ excess, variable minor $c_M$ | $pH_c = \frac{m-1}{q}(-\log c_M) + C$ | (UE19) |
| A vs. pH | Variable $c_L$ excess, constant minor $c_M$ | $pH_c = \frac{n}{q}(-\log c_L) + C$ | (UE20) |
| A vs. pH | Variable $c_M$ excess, constant minor $c_L$ | $pH_c = \frac{m}{q}(-\log c_M) + C$ | (UE21) |
| A vs. pH | Constant $c_M$ excess, variable minor $c_L$ | $pH_c = \frac{n-1}{q}(-\log c_L) + C$ | (UE22) |
| A vs. $c_L$ | Constant pH, constant minor $c_M$, variable $c_L$ excess | $\log c_L = \frac{m-1}{n}(-\log c_M) + C$ | (UE23) |
| A vs. $c_M$ | Constant pH, various minor $c_L$, constant $c_M$ excess | $\log c_M = \frac{n-1}{m}(-\log c_L) + C$ | (UE24) |

### D. Mole Ratio Method

The stoichiometry of a stable and single complex in solution can be simply determined from the brake on the absorbance vs. component concentration plot, maintaining the concentration of one component constant and letting the other one vary successively. The concentration of the metal ion is usually maintained constant. Two experimental methods are commonly possible: (1) equimolar solutions are mixed in flasks maintaining the total volume and the reaction conditions, especially pH or ionic strength constant; and (2) the concentration of the reacting component is successively increased by microliter volume changes of more concentrated solution using various types of microburettes (piston microburette), and the volume changes are neglected or corrected. The operation is directly performed in the photometric cell. The method may be considered a special case of spectrophotometric titration.[14,237-239]

For very stable complexes, the absorbance plot against the variable concentration of component forms two straight lines. The mole ratio of components results from the intersection of these lines related to the abscissa. For complexes with a large stoichiometric ratio such as $c_L/c_M > 4$, results are ambiguous. For less stable complexes, the intersection point resulting from both extrapolated tangent lines may give a correct mole ratio of the components in the complex. Serious errors appear for unstable complexes; the plot A vs. $c_L/c_M$ is exponential and does not reach the horizontal part of absorbance even when large component excesses are supplied.[136,137] Results may be improved in the presence of nonaqueous solvents or for solutions with increased ionic strength. Successive complex formation may also be indicated on the absorbance vs. the $c_L/c_M$ plot,[136,200,201] but this often causes errors in the interpretation of stoichiometry. This simple method is also suitable for the evaluation of stoichiometric coefficients in stable ternary complexes either if such complex is directly formed from the components or a mutual ligand substitution takes place.[202] For insufficiently stable complexes, the absorbance vs. mole ratio plot does not reach break and the limiting plateau until large reagent excess is applied. A function $f_{m,n}(x)$

$$f_{mn}(x) = \frac{c_L(x) - nc_M x/m}{\left\{\dfrac{x}{m(1-x)^m}\right\}^{1/n}} \qquad (65)$$

was defined for each reagent addition, where $[M_mL_n] = xc_M/m$ and $x = \Delta A/\Delta A_{0(\lim)}$. This expression has constant value as long as correct n and m are inserted into it from tested values. The conditional stability constant may also be calculated in such a case.[203]

## V. COMPUTER TESTING FOR EVALUATING COMPLEX EQUILIBRIA IN SOLUTION

At present, the treatment of spectrophotometric data by means of computer is frequently used for testing and investigation of even complicated or overlapping complex equilibria in solutions over large wavelength intervals and broad experimental conditions, i.e., concentration intervals of reacting components and pH of the solution. In this way, the most probable chemical model can be characterized or various assumed complex equilibria tested; the number of complex species, their stoichiometry, or the number of liberated protons determined; and the equilibrium or stability constants and molar absorptivities of particular complex species for a set of wavelengths evaluated. Some comprehensive literature is available describing the use of computers and computation programs for chemical model building or testing.[14,207-211] Such programs enable the determination of unknown parameters of complex equilibria, in particular the stability constants and molar absorptivities, by using minimization of least-squares sum differences between experimental and calculated absorbance values. Other programs are suitable for the calculation of distribution coefficients for particular species in solution, or various absorbance plots for curve fitting or modeling from earlier calculated, tabulated, or simulated equilibrium or stability constants and molar absorptivities.[14,15,77,79]

### A. Principles of the General Minimization Program for the Evaluation of a Chemical Model

$A_{exp}$ vs. pH, $c_L$, or $c_M$ plots measured at selected wavelengths and for a broad concentration interval of basic components (M, L, or $H^+$) supply the most suitable data for computer treatment. For the first calculation step, the stoichiometric coefficients of complex species prevailing in solution and found by simpler graphical spectrophotometric procedures or presumed per analogiam are introduced together with approximate values of their overall stability constants and molar absorptivities resulting, for example, from the particular slope-intercept transformations of equilibrium constants or limiting plateaus of particular absorbance plots.

The assumed common type of complex equilibria:

$$mM + nLH_x \rightleftarrows M_mL_nH_z + qH^+ \tag{K}$$

is usually simplified in a minimization program as

$$mM + nL + zH \rightleftarrows M_mL_nH_z \tag{L}$$

with the overall stability constant

$$\beta_{mnz} = [M_mL_nH_z]/[M]^m[L]^n[H]^z \tag{66}$$

Equations describing concentrations of basic components and the overall value of absorbance are then

$$c_M = [M] + \sum_{1}^{N} m\beta_{mnz}[M]^m[L]^n[H]^z \tag{67}$$

$$c_L = [L] + \sum_{1}^{N} n\beta_{mnz}[M]^m[L]^n[H]^z \tag{68}$$

$$c_H = [H] + \sum_{1}^{N} z\beta_{mnz}[M]^m[L]^n[H]^z \tag{69}$$

$$A_{calc,i} = \sum_{1}^{N} \epsilon_{mnz}[M_mL_nH_z] = \sum_{1}^{N} \epsilon_{mnz}\beta_{mnz}[M]^m[L]^n[H]^z \tag{70}$$

where m, n, and z are stoichiometric coefficients and N is the number of components. Using the nonlinear regression of data, the computer searches successively for the best set of parameters ($\beta_{mnz}$, $\epsilon_{mnz}$, or even m, n, and z values) for which the error square sum function U reaches its minimum:

$$U = \sum_{1}^{n} w_i(A_{exp,i} - A_{calc,i})^2 = \text{minimum} \tag{71}$$

where n is the total number of absorbance data for all solutions and all wavelengths used; the statistical weights $w_i$ are usually taken as unity. The error square sum function (Equation 71) represents an elliptic hyperparaboloid in (m + 1) dimensional space where m is the number of parameters tested. Particular programs use different minimization procedures-described in the literature[14,209] from which the pit mapping[212-214] was successfully applied in LETAGROP SPEFO[215] and derived programs[135,216,217] or the numerical differentiation by exploiting the Gauss and Newton-Raphson iteration method in absorbance matrix analysis which is characteristic for the SQUAD programs.[14,209,218-222] Due to several possible local minima besides the principal minimum of the function U, it is important to search preferentially for such a minimum for which the parameter values have a physical sense. Fully incorrectly assumed complex formation models cause divergency, cyclization, or failure of the minimization process.

The resulting minimum value of function U is further tested regarding the fit of calculated and experimental absorbance values assuming the standard deviation s(A) values belonging to the differences between calculated and experimental values of absorbance which significantly exceed the random experimental error, for all experimental solutions and wavelengths

$$s(A) = [U/(n - m)]^{1/2} \tag{72}$$

(where n is the number of experimental values and m the number of determined parameters). New complex species are introduced into the assumed model of complex equilibria successively and more or less automatically, and the calculation is recapitulated. Anomalously high values of s(A) may, however, signal a bias in the introductory data. The model with a sufficiently low s(A) value according to Equation 72 is acceptable on the whole, but particular complex species in the system are accepted as real and their parameters reliable if the corresponding standard deviations $s(\beta_{mnz})$ and $s(\epsilon_{mnz})$ are low enough and for the particular $\beta_{mnz}$ the following condition fulfilled:

$$\beta_{mnz} \geq F_0 s(\beta_{mnz}) \tag{73}$$

where $F_0 = 1.5$ at the significance level $\alpha = 0.067$.

A complex species in the system is rejected if the standard deviations of the parameters are high or the parameter values anomalous or negative, respectively (belonging to the particular complex species). In this case, the contents of such species are considered to be low in the system under given conditions. Not considering these species in minor concentrations, sometimes called computer species, may negatively influence the fitness of calculated and experimental absorbance plots, but the parameters of such species cannot be evaluated with sufficient precision by the program used. On the other hand, the computer may simulate nonexistent complex species if supplied with data charged by bias generated during experiments and measurements.

## B. Essence of the LETAGROP SPEFO Program

This minimization program (250 K) was originally developed in FORTRAN for the IBM 360/70 computer[215] and enables the spectrophotometric evaluation of complex equilibria involving or not involving proton displacements from particular $A_{exp}$ vs. $c_L$, $c_M$, or $c_H$ plots, respectively. It can also be used for the interpretation of Job curves or mole ratio plots. The error square sum is defined in terms of differences between values of measured and calculated absorbances or by least squares of relative differences according to the following:

$$U = \sum_{i=1}^{n} [(A_{exp,i} - A_{calc,i})/A_{m,i}]^2 \tag{74}$$

where $A_{m,i} = A_{exp,i}$ as far as $A_{exp,i}$ is larger than an elected limiting value, usually $A_{m,i} = 0.010$, which also may be used as a deciding factor. The criteria for an unambiguous evaluation of standard deviations $s(\beta_{mnz})$ and $s(\epsilon_{mnz})$ belonging to the estimated parameters $\beta_{mnz}$ and $\epsilon_{mnz}$ were first defined and introduced in this program.[215]

The program contains a block SPECIES SELECTOR which controls the search for the true complex equilibria. In this way, the computer automatically inserts further particular species according to a selected scheme. The species is accepted if the resulting value of U improves and the stability constant of this species fulfills the condition[212]

$$\beta_{mnz} \geqslant F_s \cdot s(\beta_{mnz}) \tag{75}$$

where $F_s$ is the so-called rejection factor, which is usually $F_s = 1$ or 1.5 (for the significance level 84 or 93.3%, respectively) if sufficiently precise data are processed, or $F_s = 3$ to 4 (significance level $\geqslant$ 99.87%) for less precise data. Thus, minor components in the system may be distinguished if precise data are available. Negative values of $\beta_{mnz}$ and $\epsilon_{mnz}$, being without physical significance, are automatically substituted by zero during minimization.

Complications were sometimes observed during the use of the first version of this program in pit mapping in the case of asymmetric form or distorted surface of the U function. Such complications were removed in the new version, the LETAGROP VRID program,[212,214] which has been the basis of the novel minimization subroutine LETAG[223,224] being used in SPEKTFOT 4[216] and SPEFO 8 (60 K)[135] programs. These programs enable the estimation of models with four or eight complex species from spectrophotometric data.

## C. Essence of the SQUAD Programs

The first version of the minimization program SQUAD (218 K) suggested in FORTRAN language[218] and for the effective estimation of complex equilibria in solution on the basis of matrix analysis of absorption spectra. Systems with five basic components — $M_1$, $M_2$, $L_1$, $L_2$, and H — may be treated assuming equilibria with simple and ternary complexes according to the following:

$$pL_1 + qL_2 + rM_1 + sM_2 \rightleftarrows (L_1)_p(L_2)_q(M_1)_n(M_2)_s + tH^+ \tag{M}$$

A number of SQUAD versions were later developed by introducing further functions and improvements. The SQUAD G version[221] is characterized by a new organization of input data for discontinuous reading of spectra at optionally selected wavelengths (no constant wavelength increment assumed). Furthermore, this version of the SQUAD program is enlarged by the calculation of the rank of the matrix of the distribution coefficients for particular species, and the segmentation of the program for small computers was also performed. The SQUAD 80 version[220] contains the error diagnosis for input data besides previous improvements and two optional algorithms, i.e., the multiple regression and nonnegative linear regression for the calculation of molar absorptivities, the latter being especially suitable for negative $\epsilon$ values. In the case of the SQUAD 81 program,[225] a subroutine, F 608, for a factorial analysis is incorporated, which enables the estimation of the number of absorbing species in solution. Subroutines for the fitness test by statistical analysis of residuals and that enabling the plotting $\epsilon$ vs. $\lambda$ graphs for particular complex species were also implemented. The recently developed SQUAD 84 version[222] represents a very general and effective program suitable for the estimation of rather complicated chemical models. There is a block DATA SIMULATION with a random numbers generator which enables the simulation of experimental plots for a selected chemical model and optional values of stability constants, molar absorptivities of particular species, and concentrations of basic components in the model. Such calculated curves corresponding to simulated experimental absorbance plots are suitable for testing the reliability of computer programs.

Besides the usual trial-and-error computation strategy during which complexes of defined stoichiometry are assumed (the integral values of m, n, and z of the $M_mL_nH_z$ complexes are maintained constant during the minimization procedure), the SQUAD 84 program enables a direct algorithmic estimation of stoichiometric indexes (ESI). This procedure consists of the simultaneous calculation of the stability constant and of particular stoichiometric indexes as real numbers instead of integers by using nonlinear regression analysis. Complex species for which the stoichiometry is estimated in this way are indicated as uncertain, in contradiction to the certain species whose stoichiometry is considered known, or at least highly probable, from previous experiments or literature. The estimated values of stoichiometric indexes are near integral values for a true assumed model. In this case, the minimization process may sometimes, however, be divergent if the stability constant and all stoichiometric indexes are simultaneously calculated for a particular complex species. This comes from some kind of interdependence of the indexes and the stability constant, but if this interdependence becomes evident by the resulting correlation coefficients, true results may be obtained by using suitable minimization strategy. Thus, the ESI procedure is useful not only for the direct estimation of stoichiometry of particular species but even for the checking of correctness of the assumed chemical model. Some kinds of plotting of various functions via computer are mentioned in Table 5.

## D. Concluding Remarks

Characteristics of selected programs are reviewed in Table 5. An advanced computer program for the interpretation of spectrophotometric data on complex equilibria in solution is composed from several logical units, each having its function in the computation, i.e., for minimization, error analysis, estimation of species number, species selector, fitness test, data simulation, and others. The structure and the description of various logical units of a program are discussed in the literature.[211]

Some proved conclusions for using computers for evaluation of spectrophotometric data on complex equilibria are collected in Table 6. Computer treatments even by using advanced computation programs may not always supply us with unambiguous results when complicated complex equilibria are established in solution. This becomes evident, for example, in similar values of s(A) being obtained for two different assumed models of complex equilibria. In

## Table 5
## SURVEY AND CHARACTERIZATION OF SELECTED PROGRAMS

| Program | Main characteristics | Ref. |
|---|---|---|
| Programs for the determination of the rank of matrix | Evaluation of the rank of absorbance matrix, i.e., of the number of absorbing species | 44, 45, 47 |
| FA 608 + EY 608 | Matrix rank and factorial analysis of absorption spectra; evaluation of stability constants and molar absorptivities of complex species | 48 |
| LETAGROP SPEFO | Evaluation of complex equilibria; determination of the number, stoichiometry, stability, and molar absorptivity of complex species from absorbance plots A vs. pH, $c_M$, or $c_L$ at selected wavelengths; criteria for omission or acceptance of complexes | 212—214 |
| SPEKTFOT 4 | Evaluation of complex equilibria, being tested for at most 4 complexes at 1 wavelength and 50 working solutions | 216 |
| SPEFO 8 | Extended version of SPEKTFOT 4; allows four more complexes to be present, with parameters $\beta,\epsilon$ remaining unchanged during minimization | 217 |
| PSEQUAD | Automatic searching for the true equilibrium model by species selector; evaluation of stability constants and molar absorptivities in the systems M-L-H, M-L-R-H, and L-H; simulation of data | 208 |
| SQUAD | Evaluation of stability constant for 6 simultaneously formed simple or ternary complex species and 10 sets of molar absorptivity values (for 4 basic and 6 new species) from absorbance data of 50 working solutions and 36 wavelengths | 218, 219 |
| SQUAD G | Enables discontinuous reading of spectra; calculation of the rank of absorbance matrix, i.e., of the number of species, and of distribution diagrams | |
| SQUAD 80 | An improved organization of input data, of error diagnosis, and of the calculation of molar absorptivities | |
| SQUAD 84 | Direct evaluation of stoichiometric indexes of complex species being considered as unknown real parameters in the minimization process (ESI approach); plotting absorption spectra in the system under various concentrations of basic components together with the calculated spectra of individual species; plotting response surface of (1 − N) values for two optional parameters; simulation of experimental plots | |
| HALTAFALL SPEFO | Calculation of distribution diagrams and of various absorbance plots for known or simulated complex equilibria (≤20 species) | |
| HALTAFALL SPEFO Graph | An extended version of HALTAFALL SPEFO permitting the plotting of some calculated plots | |

# Table 6
# SOME RULES FOR EXPLOITING COMPUTERS FOR THE SPECTROPHOTOMETRIC INVESTIGATION OF COMPLEX EQUILIBRIA

Experimental data must be of high quality and free of systematic error

A suitable choice of first tentative estimates for input data of $\beta_i$ and $\epsilon_i$ enables a reliable finding of the minimum of the U function and saves computing time

Machine computation must be suitably blended with human invention, research strategy, experience, and previous knowledge about complexation rules in solution

An effective criterion must be used to accept or reject a particular assumed complex in the complex series

A suitable general strategy during minimization must be chosen

Research results can be improved not by mathematical treatment but only by experimental improvements (purer reagents, collecting more and better experimental data, checking results by several independent methods)

such a case, some previous knowledge of complex formation or coordination rules for the particular or similar systems, as well as preliminary data resulting from graphical methods of interpretation of various absorbance plots (absorption curves; Job curves; A vs. pH, $c_M$, or $c_L$ plots; etc.) may be very useful.

The reliability of results is sometimes influenced by the more or less proper selection of wavelengths for the interpretation. Studies of the system $Ni^{2+}$ and various heterocyclic azodyes[248] have shown that if results of the computer treatment obtained by the use of the SPEFO 8[217] program for a sole wavelength (560 nm) close to the absorption maxima of studied complexes were compared with those obtained by using SQUAD G[221] for 22 wavelengths in the interval 380 to 580 nm, a better fitness for complex parameters would be obtained in the first case, in spite of the less perfect computer program used. This may be explained by the fact that the studied complexes do not become sufficiently evident in parts of the wavelength interval used for the SQUAD G program, whereas traces of impurities, colloids, parasite complexes, or competing equilibria absorb radiation more significantly, which raises the error of the computer treatment.

Examples of the successful application of computer treatment of analytical complex-forming reactions of organic reagents containing phenolic hydroxyl,[226] *o*-hydroxy-substituted *N*-heterocyclic azodyes,[14,126,128,130,134,135,227-230] *o*-hydroxy-substituted azodyes,[138,231,232] triphenylmethane dyes having salicyclic acid donor atom groups and in the presence of cationic surfactant in micellar concentration,[32] and others[14] are only mentioned here.

## VI. CONSEQUENCES FOR THE OPTIMIZATION OF SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF INORGANIC ANALYTES

Various approaches may be used to establish the optimal reaction conditions for a spectrophotometric procedure in the VIS or UV:[16,233] (1) to test successively the effects of various factors on the particular chromogenic reaction or formation of highly absorbing species by monovariant procedure, by the use of two- or three-factorial simplex[234] for evaluation of optimum conditions for the method and examine calibration absorbance function; and (2) to carry out a previous comprehensive examination of complex equilibria in solutions under broad experimental conditions, noting the evaluation of the number, stoichiometry, and formation intervals of different complex species, including tests of any competitive equilibria in solution. A successfully proved pathway for the evaluation of all necessary parameters is the combined graphical and computer treatment of spectrophotometric data. The quantitative formation interval of a single, stable, and strongly absorbing complex species of the particular analyte, with a sufficient color contrast and being formed sufficiently fast, may be read from distribution diagrams or response surfaces calculated from appropriate and previously estimated or known equilibrium or stability constants. Even preliminary or simplified calculations are useful for examining or adjusting pH and component concentrations if a single or simple equilibrium is established, or if all necessary equilibrium constants are known with sufficient accuracy. In such a case, results should be verified experimentally.[235] This way of optimization may be time consuming for complicated equilibria in solutions, but might be obligatory for developing an exact, precise, and accurate method because chemical complex equilibria are often the source of biases.

## APPENDIX I. EFFECTIVE EXPERIMENTAL APPROACHES TO OBTAINING SPECTROPHOTOMETRIC DATA

For evaluation of the complex equilibria in solution by spectrophotometric methods, the concentration of reacting components and pH must be varied in broad intervals; a sufficient number of accurate nearby experimental points without systematic errors must be available, and a sufficient number of wavelengths, including $\lambda_{max}$ of the species, selected for measurements, which is of special interest for a computer treatment.

It may not be very efficient to prepare solutions in volumetric flasks of constant volume, but there are two effective experimental methods referring to the absorbance and pH measurements under broad experimental conditions: (1) external solutions mixed by titration in a suitable reaction vessel outside the spectrophotometer and the solution transported after adjustment (pH, composition of solution) throughout a flow cell,[121,236,237] and (2) internal titration performed inside the cell by using microburettes.[237-239] During the first approach, thermostated vessels containing magnetic stirring and peristaltic pumps[236,240] or syringes were used for transportation of adjusted solution against pH and composition into the cell located in the spectrophotometer, even under inert atmosphere. Such a procedure may give erratic results if plastic material is used for tubings which seriously adsorb dye species. Glass or quartz tubings with ball-glass joints must be used in such a case.[121] The formation of small air bubbles must be avoided in the system, and longer mixing time is required when rinsing the cell and tubings after the preparation of solution. A slight adaption of the spectrophotometric compartment is usually necessary. During the second approach, the internal titration or solution preparation is directly performed in cells, and the composition of the solution is adjusted by piston microburette. A similar arrangement is frequently used for spectrophotometric titrations. Magnetic stirring is usual, and the final changes in volume may be neglected unless the change is over 1% of the total volume or corrected. In addition, a pair of electrodes (glass and reference electrodes) are also plunged into solution in the cell for equilibrium studies. Special adapters have been developed for commercial single- or double-beam spectrophotometers[187,237-239] and have been reviewed in detail in the literature.[236,238,241-243]

### Automation of Measurements

Contemporaneous microprocessed spectrophotometers are currently provided by the digital output of the signal in transmittance, absorbance, or concentration terms; automatic recording of spectra and their first and second derivatives; corrected baseline of the instrument; the automated absorbance reading at several discrete wavelength values; etc. The signal encoded in BCD code is displayed by a digital printer or type puncher; stored on magnetic type with subsequent treatment by an off-line computer; or displayed on a TV screen. The spectrophotometer may also be associated with an on-line desk computer for recording and fast treatment of data, even for select optimal instrumental parameters or for controlling its proper functions with respect to experimental conditions.

A proved system consisted of a spectrophotometer, Superscan 3 (Varian Techtron, Australia), and a desk computer, HP 9815 A, interconnected by interfaces with the instrument. A further interconnection with a precision pH meter (PHM 84, Radiometer, Denmark) and piston microburette (ABU 13, Radiometer, Denmark) might be suitable for plotting absorbances vs. pH. Absorbances were instantly treated according to a suitable program stored on magnetic tape, and the results were printed by a thermoprinter of the computer or punched on the paper type for further off-line computer treatment.[244]

A more detailed description of the present state of the automation of spectrophotometric measurements in connection with studies of complex equilibria can be found in the literature.[14] At present, there is continuous improvement in the acquisition of spectrophotometric data and automation, but biases must always be checked because they are not necessarily eliminated in this way.

## APPENDIX II. FORMAL DESCRIPTION OF COMPLEX EQUILIBRIA

Complex equilibria may be described by equilibrium, true stability, and conditional stability constants, all of which may be determined spectrophotometrically. They should be all considered as concentration constants related to a fixed ionic strength of solution.[245-247] The following equilibria are commonly established:

$$mM + nLH_x \rightleftarrows M_mL_n + xnH^+ \quad {}^*\beta_{mn} \tag{N}$$

$$mM + nLH_x \rightleftarrows M_mL_nH_z + qH^+ \quad {}^*\beta_{mnz} \tag{O}$$

$$ML_nH_z + pLH_x \rightleftarrows ML_{n+p}H_s + rH^+ \quad {}^*\beta_{n+p,s} \tag{P}$$

$$mM + nLH_x + pX \rightleftarrows M_mL_nH_zX_p + qH^+ \quad {}^*\beta_{mnzp} \tag{Q}$$

$$mM + nLH_x + pX \rightleftarrows M_mL_{n-p}X_pH_s + pLH_x + vH^+ \quad {}^*\beta_{m,n-p,p,s} \tag{R}$$

Equilibrium constants are

$${}^*\beta_{mn} = \frac{[M_mL_n][H]^{xn}}{[M]^m[LH_x]^n} \tag{76}$$

$${}^*\beta_{mnz} = \frac{[M_mL_nH_z][H]^q}{[M]^m[H_xL]^n} \tag{77}$$

$${}^*\beta_{n+p,s} = \frac{[ML_{n+p}H_s][H]^r}{[ML_nH_z][LH_x]^p} \tag{78}$$

$${}^*\beta_{mnzp} = \frac{[M_mL_nH_zX_p][H]^q}{[M]^m[LH_x]^n[X]^p} \tag{79}$$

$${}^*\beta_{m,n-p,p,s} = \frac{[M_mL_{n-p}X_pH_s][LH_x]^{p-n}[H]^v}{[M]^m[X]^p} \tag{80}$$

Stability constants are

$$\beta_{nm} = \frac{[M_mL_n]}{[M]^m[L]^n} \tag{81}$$

$$\beta_{mnz} = \frac{[M_mL_nH_z]}{[M]^m[L]^n[H]^z} \tag{82}$$

$$\beta_{mnz} = \frac{[M_mL_nH_z]}{[M]^m[L]^{n-z}[LH]^z} \tag{83}$$

$$\beta_{mnzp} = \frac{[M_mL_nH_zX_p]}{[M]^m[L]^n[H]^z[X]^p} \tag{84}$$

Successive constants are formulated in an analogous way. The stability constant of a protonated complex may be expressed in various ways.

### Conditional Stability Constants with Respect to Side Equilibria

Additional equilibria of the components being complexed, such as protonation, dissociation, hydrolysis, masking, and the effect of buffers, are taken into account in this way:

$$\beta'_{mn} = \frac{[M_mL_n]}{[M]'^m[L]'^n} = \beta_{mn}/\alpha_M^m\alpha_L^n \tag{85}$$

$$\alpha_M = [M]'/[M]; \quad \alpha_L = [L]'/[L]$$

$$[M]' = [M] + [MX] + [MX_2] + \ldots + [MX_p] \tag{86}$$

$$[M]' = [M](1 + \gamma_1[X] + \gamma_2[X]^2 + \ldots + \gamma_p[X]^p) = [M]\,\alpha_{M(X)} \tag{87}$$

$$[L]' = [L] + [HL] + [H_2L] + \ldots + [H_xL] \tag{88}$$

$$[L]' = [L](1 + [H]/K_{ax} + [H]^2/K_{ax}K_{a(x-1)} + \ldots + [H]^x/K_{ax}K_{a(x-1)} \ldots K_{a1}) \tag{89}$$

$$[L]' = [L]\,\alpha_{L(H)} \tag{90}$$

where L is the main complexing agent and X represents competing ligands, $\gamma_i$, stability constants for complexes with interferent.

The recalculation of conditional stability constants to true stability constants is more complicated if oligonuclear or ternary complexes are formed during side reactions. For more competing components in solution, the resulting side reaction coefficient $\alpha_M$ is

$$\alpha_M = \alpha_{M(X)} + \alpha_{M(Y)} + \ldots + (1 - p) \tag{91}$$

where p is the number of competing components in solution; the last term may be neglected for a large number of competing equilibria. Graphical plots of log $\alpha_{L(H)}$ vs. pH, or log $\alpha_{(M(X)}$ vs. (−log [X]) or pH, or log $\beta'_n$ vs. pH for various complexing agents are summarized in tables or graphs in published monographs.[245-247]

The total absorbance of solution (reacting components nonabsorbing) is represented by

$$A = \sum_0^z \sum_0^n \sum_0^m [M_mL_nH_z]\epsilon_{mnz} = \sum_0^z \sum_0^n \sum_0^m \beta_{mnz}[M]^m[L]^n[H]^z\epsilon_{mnz(i)} \tag{92}$$

where $\epsilon_{mnz}$, is the molar absorptivity at the ith wavelength. The concentration balance of the reacting components is

$$c_M = [M] + \sum_{1}^{z} \sum_{1}^{n} \sum_{1}^{m} m[M_mL_nH_z] \tag{93}$$

$$c_L = [L] + \sum_{1}^{z} \sum_{1}^{n} \sum_{1}^{m} n[M_mL_nH_z] \tag{94}$$

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