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Calculation of Equilibrium Constants of Ligand Binding by a Metal Ion in Solution Using a Chemometric Procedure

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Abstract—The method of calculating equilibrium constants has been considered in the work using a chemometric procedure based on the factor analysis of systems, in which a ligand added to a central ion forms in solution a number of complexes [MX_n], $n = 1–4$. Spectrophotometric titration curves were simulated by means of a matrix model of complex formation. The analysis of the simulated multivariable dependences has shown an opportunity to apply the evolutiing factor analysis to the adequate estimation of complex-formation parameters in the absence of spectral selectivity of separate spectral forms, and also in the absence of concentration regions of full domination of separate components and products of their interaction. The algorithm was developed, which allows true values of matrix model parameters determining the shape of spectrophotometric titration curves to be found without preliminary postulation of a chemical model of the system.

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The development of effective techniques for the analysis of spectroscopic dependences of solution components concentrations for the systems, in which several equilibrium reactions proceed simultaneously [2], is an urgent problem of the modern chemometrics [1]. It has been shown in a number of works that chemometric procedures based on the factor analysis [3, 4] can be successfully applied to the analysis of the data of UV and visible spectrophotometry, circular dichroism spectroscopy, and NMR spectroscopy when studying equilibria and kinetics of reactions in solutions [5–7]. The chemometric analysis of multivariable dependences is aimed at obtaining quantitative information from spectrometric data without primordial postulation of a physicochemical model of the system. The result of a “soft” chemometric modeling is a construction of dependences of the variation of abstract spectral forms concentrations defining the dispersion of a property under measurement. Concentration profiles of spectral forms can be calculated from changes in the spectra, and there is no necessity to set up a system of material balance and mass action law equations and equations connecting a measured property with an analytically determined concentration. However, there is a necessity of finding a real chemical assignment for spectral forms, i.e. of determining their chemical composition and stability. For this purpose it is necessary to assign so-called “hard” physicochemical model of the system to the abstract diagram obtained by means of the “soft” modeling, i.e. to determine forms of the complexes being in equilibrium and to calculate their stability constants. One of the first chemometric procedures used for the calculation of equilibrium constant was the evolving factor analysis (EFA) [8–10]. The combination of hard and soft modeling was used for the analysis of complex mixtures arising at a pH variation [11] and also for the calculation of step equilibrium constants [12].

The aim of the present work was to analyze the opportunity of calculating equilibrium constants for the formation of mononuclear homoligand complexes in solutions without preliminary postulation of the chemical model of a system. In the work systems were analyzed, where the concentration selectivity was not reached, i.e. there are no concentration regions of full domination of separate components and products of their interaction. As a rule, soft modeling methods do not give exact concentration profiles for such systems [13].

To check up the adequacy and possible restrictions of the calculation method using a chemometric procedure for the analysis of real experimental data, it is necessary to test it first on simulated data. To simulate various possible experimental situations, the statistical model of ligand adsorption by a central ion was used, which considers the mutual influence of adjacent ligands bound to the central ion. To describe the ligand adsorption, a number of general equations [14–18] were deduced. The model applied in the present work was described earlier [19]. In all above-enumerated methods the equations used for the complex formation description do not follow from experiments. They only allow formulating (calculating) a statistical hypothesis, which should be checked. The hypothesis is accepted if the divergence of the theory and experiment lies within the limits of an experimental error.

Simulating data matrix. According to Bouguer–Lambert–Beer's law, we shall present the experimental matrix of absorption in the matrix form $\boldsymbol{A}(N_\mathrm{p}, N_\mathrm{w})$ (N_p is the number of experimental points and N_w is the number of spectral channels) as a product of the matrix of molar extinction coefficients $\boldsymbol{E}(R, N_\mathrm{w})$ (R is the number of all spectral forms) and the matrix of concentrations $\boldsymbol{c}_\mathbf{f}(N_\mathrm{p}, R)$. The matrix of absorption can be measured experimentally only with a certain accuracy. If regular errors are excluded, elements of matrix $\boldsymbol{A}$ will contain only random errors, which were simulated in the present work by using the generator of pseudo-random numbers. As it has been shown earlier in [20], it is expedient to calculate randomized matrix $\boldsymbol{A}_i$ of "experimental" data in the form of a set of values determined with an error corresponding to normal distribution law [Eq. (1)].

$$\boldsymbol{A}_i = [\boldsymbol{c}_\mathbf{f} + \delta_{ci}][\boldsymbol{E} + \delta_{Ei}] = \boldsymbol{c}_\mathbf{f}\boldsymbol{E} + \delta_i = \boldsymbol{A} + \delta_i. \tag{1}$$

Here $\delta_{ci} = r\sigma c$, $\delta_{Ei} = r\sigma\boldsymbol{E}$, r is a random number chosen from the set with a zero mathematical expectation and a unit variance, σ is arbitrary chosen level of error, and $i = 1, 2... N$ is a number of randomization. The rank of any randomized matrix of data $\boldsymbol{A}_i$ is equal to the rank of the initial matrix of absorptions $\boldsymbol{A}$.

Matrix of concentrations. The matrix of equilibrium concentrations $\boldsymbol{c}_\mathbf{f}$ in Eq. (1) was calculated in this work on the basis of the matrix method [19]. The matrix model was offered for the description of the formation of mononuclear homoligand complexes in solutions. Briefly the essence of the model consists in the following. Let vacancies (sites) are arranged around a metal ion in fixed positions, which are capable to add ligands. In a solution these sites are occupied by solvent molecules, therefore the addition of a ligand is in fact the replacement of a solvent molecule in the internal coordination sphere. We shall admit that complexes $[\mathrm{MX}_n]$ ($n = 1, 2 \ldots N$) are formed in equilibrium conditions by free addition of ligands to the sites in various combinations. The whole set of complexes is present irrespective of concentrations of components, and the relative distribution of the central ion between the forms is defined by the stability of the corresponding compounds. Combinations and simple rearrangements give 16 possible versions of the ligand addition for the coordination number $N = 4$. Each constant $\boldsymbol{B}_k$ of the set describing stability of a corresponding complex $[\mathrm{MeX}_n]$ configuration is a product of equilibrium constants of ligand addition to separate sites β_{ki}. To calculate β_{ki}, the matrix $\boldsymbol{M}(2^N, N)$ of configurations is introduced. The matrix row $\boldsymbol{M}_k$ reflects a possible configuration of the complex with a sequence of zeros in positions where sites are free and with a sequence of units in positions where sites are occupied by ligands. The equilibrium constant of a ligand addition to a certain site can be presented as a product [Eq. (2)].

$$\overline{\beta}_{\boldsymbol{ki}} = (\boldsymbol{M}_{\boldsymbol{ki}}\bar{K}\omega_{ki})_{M_{ki}\neq 0}. \tag{2}$$

Here $K = K_\mathrm{in}$ is the internal equilibrium constant of the reaction of the first ligand addition to the central ion and ω_{ki} is the correction for the mutual influence, which generally depends on the coordination polyhedron geometry. The present work considers examples resulted in the formation of a tetrahedral complex. In case of this geometry sites are equivalent ($\omega_{cis} = \omega_{trans}$) and, hence, the formation function will be defined by two parameters K_in and ω. In case of the square-planar D_{4h} coordination of the polyhedron geometry, three independent variables K_in, ω_{cis}, and ω_{trans} are necessary. The matrix model also can be applied to the description of octahedral complexes if the equatorial plane of a complex is filled independently with a subsequent coordination of ligands in axial positions of the octahedron. In this case five independent variables K_in, ω_{cis}, ω_{trans}, K_5, and K_6 are necessary for the model. It is sufficiently correct to consider the addition in axial positions as being independent if an appreciable formation of forms occurs in the region of much differing concentrations.

$$\boldsymbol{c}_\mathrm{form} = \left[[\mathrm{Me}], \left[[\mathrm{Me}]\times\boldsymbol{B}_{\boldsymbol{k}}\times([\mathrm{X}]^S)^T\right]^T\right]\cdot\boldsymbol{P}. \tag{3}$$

To summarize concentrations of $[\mathrm{MeX}_n]$ with the same number of coordinated ligands in various configurations, we shall introduce matrix $\boldsymbol{P}(2^N, 5)$. Elements of this matrix are equal to unit in the columns corresponding to occupied sites, whereas the other elements are equal to zero. Now, using Eq. (3), we can calculate the matrix of equilibrium concentrations of $[MeX_n]$ forms for particular equilibrium concentrations of the ligand and the complex-forming ion.

$$[\mathrm{Me}] = \frac{\boldsymbol{c}_{\mathrm{Me}}}{1 + [\mathrm{X}]^{\boldsymbol{S}}\boldsymbol{B}_{\boldsymbol{k}}}. \tag{4}$$

Here $\boldsymbol{S} = \Sigma\boldsymbol{M}$ is the sum of elements of an $\boldsymbol{M}$ matrix raw; × and · signs of the elementwise and usual multiplication of matrices. Further equation (3) will be used to calculate a ligand equilibrium concentration at specified total concentrations c_{X} and c_{Me}, which is necessary for iterative mathematical procedures of processing spectrophotometry data by the least-squares method. The equilibrium concentration of the central ion is calculated by formula (4).

The matrix c_{form} is a matrix of concentrations of forms usually used in the stepwise complex-formation model. For example, for a system Me^{2+}–L it is the matrix of concentrations of the forms $[\mathrm{Me}]^{2+}$, $[\mathrm{MeL}]^{2+}$, $[\mathrm{MeL}_2]^{2+}$, $[\mathrm{MeL}_3]^{2+}$, and $[\mathrm{MeL}_4]^{2+}$ at specified total concentrations c_{X} and c_{Me}. The use of Eq. (4) allows us to calculate the matrix of concentrations c_{f} of forms for a full set of concentrations of solution components $[c_{\mathrm{X}}(1), c_{\mathrm{Me}}(1); c_{\mathrm{X}}(2), c_{\mathrm{Me}}(2); \ldots]$.

Matrix of molar extinction coefficients. Spectra of arbitrary chosen Gauss forms were used for modeling the matrix of molar extinction coefficients. A spectrum of a form was calculated by formula (5).

$$E = \frac{e^{-(X-\mu)^2/2\sigma^2}}{\sigma\sqrt{2\pi}}. \tag{5}$$

Here X is an arbitrary set of numbers from the interval 1–10.

Chemometric data analysis. Calculation of abstract concentration profiles from a matrix of spectrophotometric data. The aim of the chemometric analysis is to solve the inverse problem: to find matrixes of concentration and coefficients of molar absorption from the matrix of experimental absorptions. For this purpose first of all it is necessary to determine the number of principal factors equal to the number of forms of compounds in an equilibrium mixture, the interaction of which causes the absorption variance. The procedure of singular decomposition allows us to reproduce the ith matrix of data by means of a fixed number of new variables, which are a linear combination of initial variables [Eq. (6)].

$$\boldsymbol{A}_{\boldsymbol{i}} = \boldsymbol{USV}^{\boldsymbol{T}} + \tau_i = \boldsymbol{A}_{\boldsymbol{i}}^{*} + \tau_i. \tag{6}$$

Here $\boldsymbol{U}$ and $\boldsymbol{V}^T$ are orthogonal matrixes, $\boldsymbol{S}$ is the diagonal matrix with the number of nonzero diagonal elements equal to the rank of matrix $\boldsymbol{A}_i$ (equal to the number of principal factors), $\boldsymbol{A}_i^{*}$ – $\boldsymbol{A}_i$ the matrix reproduced by means of R factors, and τ_i is a reproduction error. The number of principal factors is selected in such a way that the reproduction error τ_i is less than or equal to an experimental error ($\tau_i \leq \delta\boldsymbol{A}_i$). In the present work R was calculated using the method of principal components analysis (PCA) [4]. The found R value is a key for the further mathematical modeling the equilibrium in the system. In the present work the examples of situations arising upon an acido complex formation were considered. If n molecules of a solvent in the internal coordination sphere are replaced by an acido ligand, as a rule, strong charge-transfer bands appear in the spectrum. Therefore the variation of the spectrum is caused by the variation of the concentrations of the aqua ion and n acido complexes. In this case $R = n + 1$. When one acido ligand is replaced by another, compounds $[\mathrm{MY}_{m-n}\mathrm{X}_n]$ (here $R = m + 1$) will be absorbing forms. The rank of the matrix is equal to the number of the linear variables necessary for the description of observable experimental dependences at each wavelength of the measurement. The number of nonlinear parameters can be calculated by means of mathematical modeling, proceeding from the chemical nature of the system. It is difficult to specify a correct initial approach for a set of independent variables directly from experimental spectrophotometric data. An incorrect initial approach, which is overstepping the limits of a convergence area, obviously leads to the incorrect solution that can be one of modeling error reasons. Therefore, to minimize the uncertainty of the initial approach choice, it is necessary to apply chemometric procedures free from modeling restrictions. One of the first methods used for the calculation of concentration profiles from spectrophotometric data was the soft modeling EFA method, which was applied in the present work to calculate the initial approach c_i. The algorithm of the EFA procedure [8–10] consists in the calculation of the eigenvalue of the covariant matrix $\boldsymbol{A}_{\boldsymbol{k}}$ constructed from j rows of the experimental data matrix as a function of the number of rows [Eq. (7)].

$$\boldsymbol{A}_{\boldsymbol{k}} = \boldsymbol{A}_{\boldsymbol{i}}^{\boldsymbol{T}}(j, N_{\mathrm{w}})\boldsymbol{A}_{\boldsymbol{i}}(j, N_{\mathrm{w}}). \tag{7}$$

Varying j from $R + 1$ up to N_{p} in the direct, and then in the reverse course, we constructed the diagram of variation of (unnormalized) concentrations of R abstract forms as functions of a row number in the data matrix. The normalization on the total analytical concentration of a titrated compound gives the initial approach of the matrix c_i. The use of this approach in iterative fitting procedures serves for the calculation of c_f and $\boldsymbol{E}$ values corresponding to the Eq. (1) solution. Because of rotary uncertainty, the calculated concentration profiles deviate from true profiles. For the systems, in which variations of spectra are caused by a displacement of equilibrium owing to complex formation of non-polymeric components, the effective way, allowing rotary uncertainty to be removed, is imposing of restrictions on the unimodality and nonnegativity of concentration profiles on the forms of profiles.

Iterative optimization procedure (calculation of equilibrium constants). In the present work the standard Levenberg–Marquardt's algorithm of the nonlinear least squares method (NLSM) [21, 22] was used to calculate parameters of the matrix model. The normalized sum of squares of deviations of experimental absorption values $\boldsymbol{A}_{\mathrm{exp}}$ from corresponding calculated values $\boldsymbol{A}_{\mathrm{calc}}$ [Eq. (8)] was chosen as the function to be minimized.

$$\boldsymbol{S} = \boldsymbol{\Sigma}(\boldsymbol{A}_{\mathrm{exp}} - \boldsymbol{A}_{\mathrm{calc}})^2. \tag{8}$$

The value of $\boldsymbol{A}_{\mathrm{calc}}$ was calculated in each iterative step by formula (9) with current values of model parameters.

$$\boldsymbol{A}_{\mathrm{calc}} = [\boldsymbol{A}_{\mathrm{exp}}/c_f]_{>0}c_f. \tag{9}$$

Here $[\ldots]_{>0}$ is the operator cutting out physically meaningless negative values, c_{f} is the matrix of spectral forms concentrations constructed from a row of matrix elements c_{form} or from their sums. The deviation of calculated values from experimental values was calculated in a matrix form by Eq. (10).

$$\boldsymbol{PE}(\boldsymbol{A}_{\mathrm{exp}},\boldsymbol{A}_{\mathrm{calc}}) = \left(\frac{\mathrm{Trace}[\boldsymbol{A}_{\mathrm{exp}} - \boldsymbol{A}_{\mathrm{calc}})(\boldsymbol{A}_{\mathrm{exp}} - \boldsymbol{A}_{\mathrm{calc}})^T]}{\mathrm{Trace}[\boldsymbol{A}_{\mathrm{exp}}\boldsymbol{A}_{\mathrm{calc}}{}^T]}\right)^{1/2}. \tag{10}$$

Adequacy of the hypothetic model was checked by comparison of experimental and calculated absorption variances [1]. Tabulated values of Fisher's criterion F_{tabl} (α 0.05) [23] were used in the check. According to the principle of maximal likelihood, under the condition of homogeneity of variance values, the parameters corresponding to the minimum of the $\boldsymbol{PE}$ function correspond to the most probable model of a system. In the examples considered in the present work the most probable model coincides with the model used for the simulation.

Calculations with the simulated data as an example. The formation of a sufficiently stable complex of a ligand with a central ion allows us to create without difficulty experimental conditions of the concentration selectivity for both low and high concentrations. In the region of low ligand concentrations a solvated central ion dominates, whereas on addition of a small ligand excess a saturated complex is formed. Using the scheme described above, the matrix ***A1*** of absorptions was simulated by Eq. (1) for a system, in which a stepwise ligand addition to the central ion is governed by the matrix model of complex formation.

The result of the decomposition of the absorption matrix ***A1*** simulated by means of the EFA procedure for the system with anticooperative ligand addition (theoretical values of $\log K_{\mathrm{in}}$ 3.18 and of ω 0.35) is given in Fig. 1. The replacement of water by a chloride ion in the internal coordination sphere of Pd(II) in an aqueous solution [24] can be presented as an experimental example of such system. The principle component analysis of the absorption matrix ***A1*** shows that five components describe 100% of a variance, i.e. the rank of the matrix is equal to five (see the table). It is seen from Fig. 1 that in this case points of crossing plotted lines calculated by EFA are close to points of crossing dependences of concentrations of the forms on the equilibrium ligand concentration corresponding to the matrix model. The results obtained allow us to estimate rather precisely the required constant of the ligand addition to the site of the complex-forming agent by formula (11).

$$-\log [\mathrm{L(Nt)}\cdot 4] = \log K^{*}_{\mathrm{in}}. \tag{11}$$

Here L(Nt) is the value of equilibrium concentration in the point of crossing lines corresponding to the relative contents of two first spectral forms in the equilibrium mixture, as calculated by means of EFA.

The value of the equilibrium concentration in the point of crossing these lines in the case under consideration is log L(Nt) –4.1, which gives the estimate of the internal constant $\log K^{x}_{\mathrm{in}}$ by formula (11) at 3.50. The accuracy of the found estimate is quite sufficient for the subsequent correct refining by NLSM (convergence to a true minimum). The second parameter of the matrix model can be estimated from the value of the ratio of the first and second stepwise addition constants. This ratio is independent of absolute values of stepwise constants. It is seen from Fig. 1 that the values of equilibrium concentration L in

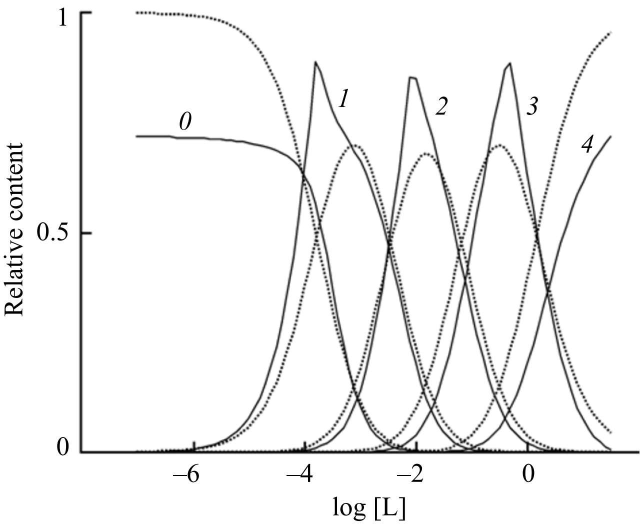

Fig. 1. Relative contents of complexes (*0*) [Me], (*1*) [MeL], (*2*) [MeL_2], (*3*) [MeL_3], and (*4*) [MeL_4] in relation to the logarithm of the ligand equilibrium concentrations calculated by means of EFA. Contents of complexes calculated according to the model, K_{in}, is designated by dotted lines.

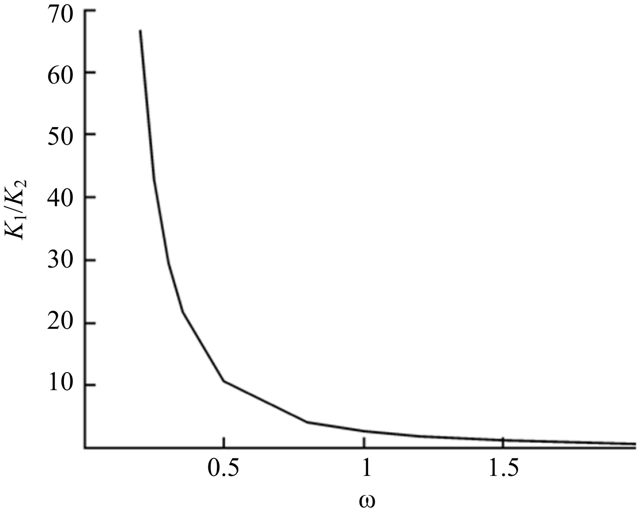

Fig. 2. Dependence of the ratio of stepwise complex-formation constants on the mutual interaction parameter.

the point of crossing lines corresponding to the relative contents of the second and third spectral forms in the equilibrium mixture calculated by means of EFA practically coincide with the value of the equilibrium L concentration, at which relative fractions of [MeL] and [MeL_2] forms calculated according to the theoretical model are equal. This correspondence allows us to determine the near-true value of parameter ω from the correlation diagram presented in Fig. 2.

Thus initial approaches for two parameters of the matrix model have been found. The accuracy of the found estimate is quite sufficient for correctness of the subsequent refining by NLSM (convergence to a true minimum). The result obtained testifies that the fulfilled mathematical processing of experimental data without primordial postulation of a system chemical model allows us to find true values of the first ligand addition constant and the mutual influence parameter preset for simulating spectrophotometric titration curves. However, it is necessary to note that the above estimation is possible only if an equilibrium ligand concentration has been determined experimentally. Such concentration can be measured, for example, by an ion-selective electrode when studying the complex formation with halide ions. If equilibrium concentra-

Results of the analysis of simulated data matrixes by the principal components method

Principal component no.	Matrix eigenvalue		Variance described by principal component, %		Variance described by the sum of principal components, %	
	A1	***A2***	***A1***	***A2***	***A1***	***A2***
1	5.33×10^{-1}	7.90×10^{-1}	59.89	75.13	59.89	75.13
2	2.09×10^{-1}	1.78×10^{-1}	23.43	16.92	83.32	92.06
3	1.08×10^{-1}	6.90×10^{-2}	12.11	6.55	95.43	98.61
4	3.46×10^{-2}	1.41×10^{-2}	3.89	1.34	99.32	99.95
5	6.08×10^{-3}	5.19×10^{-4}	0.68	0.05	100.0	100.0
6	3.59×10^{-17}	5.75×10^{-17}	0.0	0.0	100.0	100.0

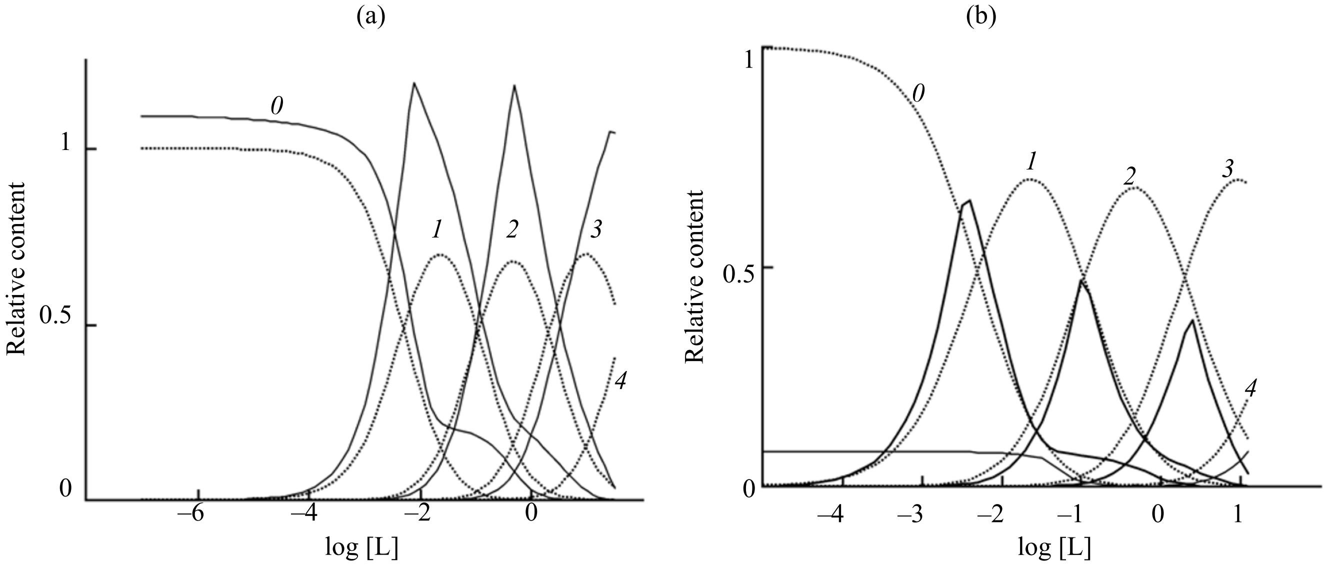

Fig. 3. Relative contents of complexes as functions of the logarithm of ligand equilibrium concentration (*0*) [Me], (*1*) [MeL], (*2*) [MeL_2], (*3*) [MeL_3], (*4*) [MeL_4]) calculated by means of EFA; (a) four factors; (b) five factors. Content of complexes calculated according to the model is designated by dotted lines, K_{in} 50; ω 0.35.

tions of system components are unknown, it is necessary to take into account a shift between the total concentration and the equilibrium concentration of a ligand when estimating parameters of the model.

If the substance which is the source of the ligand does not possess sufficient solubility, a situation arises which does not allow achieving a complete complex formation by a central ion in a solution. This situation is most prob-able, if a ligand and a complex-forming agent give rise to insufficiently stable complexes. The formation of bromide complexes in the system copper(II)–bromide ions in dimethyl formamide [25] was chosen in the present work as an experimental example. In this case the central ion forms an unstable complex with the ligand (the values of log K_{in} 1.70 and ω 0.35 were used for the simulation). The matrix of absorptions ***A2*** was simulated by means of Eq. (1) according to the scheme described above. The diagram of relative content of spectral forms found by the decomposition of the matrix ***A2*** by means of the EFA procedure is shown in Fig. 3.

In this case we consider the situation without concentration selectivity in the region of high ligand concentrations (one-sided concentration selectivity). The analysis of the simulated matrix of absorptions ***A2*** by the method of principal components points to the fact that 100% of the variance describes five components, i.e. the matrix rank is five (see the table). Therefore five possible spectral forms were considered originally when carrying out the EFA procedure. The result of the decomposition is shown in Fig. 3. However, it is seen that only 0.05% of the total variance falls to the share of the fifth component (see the table). A noticeable contribution of this factor is shown only at high concentrations. In real experimental conditions in the presence of instrument noise and unaccounted factors causing an additional variance (impurities etc.) the contribution from a given component can appears within the experimental error region. Therefore the factor analysis was fulfilled taking into account only four spectral forms. The result of the decomposition is shown in Fig. 3a. Comparing the results of these decompositions, we can see that in calculations taking into account four forms points of crossing the lines calculated by means of EFA are close to points of crossing of concentration dependences of the forms for the equilibrium ligand concentration, and when five forms are considered these points are close to the maxima in EFA plots. The value of the logarithm of equilibrium concentration in the point of crossing lines describing relative contents of first two spectral forms and constructed by means of EFA is in this case –2.5. According to formula (11), this value estimates the internal constant log K_{in}^{x} at 1.90. The accuracy of this estimate is quite sufficient for correct subsequent refining parameters of the model by NLSM, which confirms the correctness of the fulfilled mathematical processing of the spectrophotometric titration data. When not so stable complexes are formed the values of equilibrium concentrations are not too different from the values of the total concentrations, therefore the distinction between the total and equilibrium concentrations can be not considered in the estimation of the internal formation constant in the example at hand.

In conclusion it is possible to note that the foregoing research has shown that the chemometric procedure based on the evoluting factor analysis EFA can be used for the calculation of initial approximations of the matrix model parameters of complex formation in solution, namely a constant of the first ligand addition and the parameter of mutual influence between added ligands, from spectrophotometry data, as shown by the examples of typical situations taking place in real systems. The developed procedure allows true values of equilibrium constants at incomplete concentration selectivity to be determined after refining model parameters by the least-squares method.

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