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Number of species in complexation equilibria of SNAZOXS or Naphtylazoxine 6S and Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} ions by PCA of UV–vis spectra

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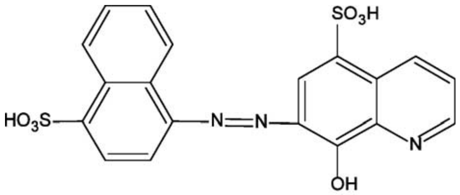
Abstract

A critical comparison of the various PCA methods on the absorbance matrix data concerning the complexation equilibria between SNAZOXS and Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} or Naphtylazoxine 6S and Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} at 25 °C is performed. The number of complex species in a complex-forming equilibria mixture is the first important step for further qualitative and quantitative analysis in all forms of spectral data treatment. Therefore, the accuracy of the nine selected index functions for the prediction of the number of light-absorbing components that contribute to a set of spectra is critically tested using the principal component PCA algorithm INDICES in S-Plus software. Four *precise methods* based upon a knowledge of the experimental error of the absorbance data and five *approximate methods* requiring no such knowledge are discussed. Precise methods always predict the correct number of components even a presence of the minor species in mixture. Due to the large variations in the index values and even at logarithmic scale they do not reach an obvious point where the slope changes. An improved identification with the second or third derivative and derivative ratio function for some indices is preferred. Behind the number of various complexes formed the stability constants of species ML, ML_2, (and ML_3, respectively) type $\log \beta_{11}$, $\log \beta_{12}$, (and $\log \beta_{13}$, respectively) for the system of SNAZOXS (ligand L) with six metals (the standard deviation $s(\log \beta_{pq})$ of the last valid digits are in brackets) Cd^{2+} (4.50(3), 8.36(7)), Co^{2+} (5.75(6), 9.79(9), 13.05(2)), Cu^{2+} (6.69(6), 11.40(7)), Ni^{2+} (6.44(8), 10.91(11), 15.07(10)), Pb^{2+} (5.63(5), 9.97(9)) and Zn^{2+} (5.11(3), 8.84(5)) and for system of Naphtylazoxine 6S with Cd^{2+} (6.08(4), 11.44(7), 16.06(11)), Cu^{2+} (7.80(8), 13.41(14)), Ni^{2+} (6.35(12), 11.43(19), 16.68(24)) and Zn^{2+} (7.01(8), 12.65(15)) at 25 °C are estimated with SQUAD(84) nonlinear regression of the mole-ratio spectrophotometric data. The proposed strategy of an efficient experimentation in a stability constants determination, followed by a computational strategy, is presented with goodness-of-fit tests and various regression diagnostics able to prove the reliability of the chemical model proposed.

Keywords: Principal component analysis; Decomposition of absorbance matrix; Factor analysis; Rank of matrix; Number of species; Number of components; Error of spectrophotometer

1. Introduction

The protonation and complex-forming equilibria of some sulphoazoxine oligomers have been studied systematically in our laboratory [1–11]. The protonation constants and the number of oligomer species for two 7-arylazo derivatives of 8-hydroxyquionoline-5-sulphonic acid differing in the steric arrangement of their molecules, viz. SNAZOXS and Naphtylazoxine 6S were studied [1,7,8,10].



SNAZOXS

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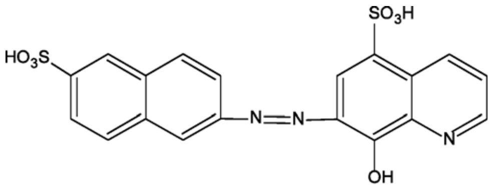


Naphtylazoxine 6S

The two substances are frequently applied as metalochromic indicators in analytical practice, as they form complex compounds, usually yellow in color, with a series of metals [1]. SNAZOXS or 7-(4-sulpho-1-naphtylazo)-8-hydroxyquinoline-5-sulphonic acid forms stable complexes with copper(II) ions; somewhat weaker complexes are formed with nickel(II) and cobalt(II) ions. Protonation equilibria of Naphtylazoxine 6S or 7-(6-sulpho-2-naphtylazo)-8-hydroxyquinoline-5-sulphonic acid have previously been studied [1,7,8,10] while the complex-forming equilibria and the stability constants of these two sulphoneazoxines with various metal cations are presented in this paper. Such analysis is, in general, performed in several steps: in the first step the number of components is determined, and then concentrations of all of the complex species involved are calculated. Several instrumental methods have been devised for the second step, and depend on the chemical model of all equilibria and experimental design. Predicting the number of components is pertinent to all methods of chemical model determination.

Determining the number of complex species in a mixture with the use of spectroscopic data is a challenging problem. It forms the first step for further qualitative and quantitative analysis in all forms of spectral data treatment. Procedures for determining the chemical rank of a matrix concerning a variety of empirical and statistical methods based on principal component analysis (PCA) have been reported and critically compared [12–18]. Using PCA, a set of correlated variables is transformed into a set of uncorrelated variables, principal components such that the first few components explain most of the variation in the data. PCA will extract some of the noise i. e. experimental and/or random error which will usually be represented by the principal components with the smallest sizes or variances. In the study of complex-forming equilibria, for instance, a reliable determination of the number of components involved will help to obtain a reasonable interpretation of various complexes.

The purpose of this study is to make a critical comparison of the various PCA methods on the absorbance matrix data concerning the complex-forming equilibria of two sulphoneazoxines with six metal cations.

2. Theoretical

Principal component analysis has been used in a powerful fashion for determining the number of components that contribute to the absorption spectra of multicomponent systems [12,18].

2.1. Notation

The absorbance $A_{i,j}$ is the element of the absorbance matrix $\boldsymbol{A}$ of size $n \times m$ being measured for n solutions with known total concentrations of 3 basic components, c_{M}, c_{L} and c_{H}, at m wavelengths. Throughout this work, it is assumed that the $n \times m$ absorbance data matrix $\boldsymbol{A} = \varepsilon \boldsymbol{C}$ containing the n recorded and digitized spectra as rows can be written as the product of the $m \times n_{\mathrm{c}}$ matrix of molar absorptivities $\boldsymbol{\varepsilon}$ and the $n_{\mathrm{c}} \times n$ concentration matrix $\boldsymbol{C}$. Here n_{c} is the number of components that absorb in the chosen spectral range. The rank of the matrix $\boldsymbol{A}$ is obtained from the equation $\mathrm{rank}(\boldsymbol{A}) = \min[\mathrm{rank}(\boldsymbol{\varepsilon}), \mathrm{rank}(\boldsymbol{C})] \leq \min(m, n_{\mathrm{c}}, n)$. Since the rank of $\boldsymbol{A}$ is equal to the rank of $\boldsymbol{\varepsilon}$ or $\boldsymbol{C}$, whichever is the smaller, and since $\mathrm{rank}(\boldsymbol{\varepsilon}) \leq n_{\mathrm{c}}$ and $\mathrm{rank}(\boldsymbol{C}) \leq n_{\mathrm{c}}$, then provided m and n are equal to or greater than n_{c}, it will only be necessary to determine the rank of matrix $\boldsymbol{A}$ which is equivalent to the number of dominant light-absorbing components [16–18].

2.2. Determination of the number of complex species

All spectra evaluation were performed in the S-Plus programming environment with the INDICES algorithm [18,19]. Most index methods are functions of the number of $PC(k)$ into which the spectral data usually are plotted against k, $PC(k) = f(k)$, and when the $PC(k)$ reaches the value of the experimental error of spectrophotometer used, the corresponding k represents the number of light-absorbing components in a mixture, $n_{\mathrm{c}} = k$. The dependence $PC(k) = f(k)$ decreases steeply with an increasing number of PCs as long as the PCs are significant. When k is exhausted the indices fall off, some even displaying a minimum. At this point $n_{\mathrm{c}} = k$ for all index functions. The index values at this point can be predicted from the properties of the noise, which may be used as a criterion to determine n_{c}, cf. ref. [12,18]. The various indicator function $PC(k)$ techniques developed to deduce the exact size of the true component space can be classified into two general categories: (a) precise methods based upon a knowledge of the experimental error of the absorbance data, and (b) approximate methods requiring no knowledge of the experimental error [17]. Elbergali et al. [16] proposed a modification of index methods using derivatives to improve identification of the number of components. The *second derivative criterion* $SD(k)$ is based on the point where the slope changes and reaches a maximum. The $SD(k)$ is defined $SD(k) = \log[PC(k+1)] - 2 \times \log[PC(k)] + \log[PC(k-1)]$ and $n_{\mathrm{c}} - k$ should be at the first maximum of the $SD(k)$ function, where PC stands for an index function. The *third derivative* $TD(k)$ value crosses zero and reaches a negative minimum which can be used as criterion. The $TD(k)$ is defined as $TD(k) = \log[PC(k+2)] - 3 \times \log[PC(k+1)] + 3 \times \log[PC(k)] - \log[PC(k-1)]$ and n_{c} should be equal to k value where $TD(k)$ has its first minimum. The change in slope can also be found by calculating the *derivatives ratio* $ROD(k)$ by $ROP(k) = \{PC(k-1) - PC(k)\}/\{PC(k) - PC(k+1)\}$.

Ideally, *ROD*(k) should have a maximum at the point where $k = n_c$.

2.2.1. Precise indices

Precise indices methods are based on a comparison of an actual index of the method used with the experimental error of the instrument used. Most of the techniques presented are empirical functions based on eigenvalues of the absorbance matrix [18,20]. The first n_c eigenvalues, being called a set of primary eigenvalues contain contributions from the real components and should be considerably larger than those containing only noise. The second set called the secondary eigenvalues, contains $(m - n_c)$ eigenvalues and they are referred to as non-significant eigenvalues.

(1) *Kankare's residual standard deviation*, $s_k(A)$: The values $s_k(A)$ for different number of components k are plotted against an index k, $s_k(A) = f(k)$, and the number of significant components is an integer $n_c = k$ for which $s_k(A)$ is close to the experimental error of absorbance $s_{inst}(A)$, [18,21].

(2) *Residual standard deviation*, *RSD*(k), is used analogously as in the previous $s_k(A)$ method. Both, $s_k(A)$ and *RSD* represent excellent criteria for deducing which eigenvalues belong to the primary and secondary sets. If the *RSD* is greater than the estimated experimental error of absorbance RSD_{inst}, an sufficient number of components k has been selected [12,18].

(3) *Root mean square error*, *RMS*(k) is closely related to *RSD* even thought they measure two entirely different errors. *RMS* measures the difference between raw data and PC-regenerated data. *RSD* measures the difference between raw data and pure data possessing no experimental error [12].

(4) The *average error criterion*, *AE*(k), is used analogously as in the previous $s_k(A)$ method, [12,18]. Eigenvectors are systematically added to the scheme until the calculated *AE* approximately equals the estimated experimental error, AE_{inst}.

(5) The *Bartlett* χ^2 *criterion*, $\chi^2(k)$ is used so that the true number of significant components corresponds to the first k value, for which $\chi^2(k)$ is less than critical $\chi^2(k)_{expected} = (n - k)(m - k)$. This method takes into account the variability of the error from one data point to the next. Its disadvantage is that one must have a reasonably accurate error estimate for each data point, [12,18].

2.2.2. Approximate methods

A more difficult problem is to deduce the number of components without relying on an estimation of the experimental error of absorbance.

1. *Exner function* $\psi(k)$*:* The Exner $\psi(k)$ function may be used for identifying the true dimensionality of data. Exner proposed that $\psi = 0.3$ can be considered a fair correlation, $\psi = 0.2$ can be considered a good correlation and $\psi = 0.1$ an excellent correlation. It means that for $\psi < 0.1$ the corresponding k can be taken as the number of light-absorbing species in solution.
2. *Scree test*, *RPV*(k)*:* The Scree test for identifying the true dimensionality of a data set, proposed by Cattell [22], is based on the observation that the residual variance should level off before those dimensions containing random error are included in the data reproduction. The point where the curve begins to level off, or where a discontinuity appears, is taken to be the dimensionality of the data space [12,22].
3. *Imbedded error function*, *IE*(k)*:* The imbedded error is a function of the error eigenvalues. The behavior of the *IE*(k) function, as k varies from 1 to m, can be used to deduce the true dimensionality of the data. The *IE*(k) function should decrease as the true dimensions are used in the data reproduction. However, when the true dimensions are exhausted, and the error dimensions are included in the reproduction, the *IE*(k) should increase.
4. *Factor indicator function*, *IND*(k)*:* The factor indicator function *IND*(k) is an empirical function which appears more sensitive than the *IE*(k) function in identifying the true dimensionality of an absorbance data matrix [12]. This function, like the *IE*(k) function, reaches a minimum when the correct number of latent variables or k PC dimensions have been employed in the data reproduction. However, it has been seen that the minimum is more pronounced and can often occur in situations where the *IE*(k) function exhibits no minimum.

2.3. Determination of the chemical model in complex-forming equilibria

Computations related to the determination of stability constants were performed by regression analysis of spectra. If the complex-forming equilibria between a metal, M, (the charges are omitted for the sake of simplicity), a ligand, represented by the anion, L, of sulphoneazoxine and a proton, H, is considered to form a set of species which have a general formula $M_pL_qH_r$ in a particular *chemical model* and are represented by n_c, the number of species, $(p, q, r)_i$, $i = 1, \ldots, n_c$, their stoicheiometry, then the overall stability constant, β_{pqr}, may be expressed as

$$\beta_{pqr} = \frac{[M_pL_qH_r]}{[M]^p[L]^q[H]^r} \tag{1}$$

where free concentration [M], [L], [H] and $[M_pL_qH_r]$. For the ith solution measured at the jth wavelength, the absorbance, $A_{i,j}$, is defined as:

$$A_{i,j} = \sum_{n=1}^{n_c} \varepsilon_{j,n}[M_pL_qH_r]_n = \sum_{n=1}^{n_c} (\varepsilon_{pqr,j}\beta_{pqr}[M]^p[L]^q[H]^r)_n \tag{2}$$

where $\varepsilon_{pqr,j}$ is the molar absorptivity of the $M_pL_qH_r$ species with stoichiometric coefficients p, q, r measured at the jth wavelength. The multi-component spectra analysing program SQUAD(84) [23–26] can adjust $\boldsymbol{\beta}_{pqr}$ and $\boldsymbol{\varepsilon}_{pqr}$ for absorption spectra by minimising the residual-square sum, U,

$$U=\sum_{i=1}^{n}\sum_{j=1}^{m}(A_{\mathrm{exp},i,j}-A_{\mathrm{calc},i,j})^2 =\sum_{i=1}^{n}\sum_{j=1}^{m}\left(A_{\mathrm{exp},i,j}-\sum_{k=1}^{n_c}\varepsilon_{j,k}[\mathrm{M}_p\mathrm{L}_q\mathrm{H}_r]_k\right)^2 = \text{minimum} \qquad (3)$$

where $A_{i,j}$ represents the element of the experimental absorbance response-plane of size $n \times m$ and the independent variables are the total concentrations of the basic components c_{M}, c_{L} and c_{H} being adjusted in n solutions. The unknown parameters of a chemical model may be divided into two equal groups: (1) a hypothetical chemical model being supplied by the user should contain (a) a guess as to the number of light-absorbing species in solution, n_{c}, and (b) a list of species stoichiometry indices $(p, q, r)_i$, $i = 1,\ldots, n_{\mathrm{c}}$; (2) the best estimates of the stability constants, $\beta_{pqr,i}$, $i = 1,\ldots, n_{\mathrm{c}}$, are adjusted by the SQUAD(84) regression algorithm. At the same time a matrix of molar absorptivities $(\varepsilon_{pqr,j}, j = 1,\ldots, m)_k$, $k = 1,\ldots, n_{\mathrm{c}}$, as non-negative reals is estimated, based on the current values of stability constants. For a set of current values of $\beta_{pqr,i}$, the free concentrations of metal [M] and ligand [L], ([H] being known from pH measurement), for each solution is calculated, and then the concentrations of all the complexes in equilibrium mixture $[\mathrm{M}_p\mathrm{L}_q\mathrm{H}_r]_j$, $j = 1,\ldots, n_{\mathrm{c}}$, forming for n solutions the matrix $\boldsymbol{C}$. The experimental and computation scheme for the determination of the stability constants of a multi-component system was taken from Meloun et al. [25,26]. Various hypotheses of chemical models with refined parameters have been tested and the statistical characteristics describing the test-of-fit of regression spectra through experimental points have been calculated. The calculated standard deviation of absorbance $s(A)$ and the Hamilton *R-factor* were used as the most important criteria for a fitness test. If after termination of the minimization process the condition $s(A) \approx s_{\mathrm{inst}}(A)$ is met and the *R-factor* is less than 1%, the hypothesis of the chemical model is taken as the most probable and is accepted [26,27].

3. Experimental

3.1. Chemicals and solutions

SNAZOXS and Naphtylazoxine 6S of analytical-reagent grade were obtained from Spolana (Neratovice, Czech Republic) and purified from a concentrated solution with acetone as described previously [10]. The actual concentration of sulphoazoxine was determined by emf titration with NaOH and evaluated by regression program analysis with the ESAB [28] and MAGEC [29] programs. In this way, the purity of the indicators was found to be SNAZOXS 97.3%, Naphtylazoxine 6S 78.3%. The impurities were mostly inorganic salts.

Perchloric acid, 1 M, was prepared by dilution of concentrated $\mathrm{HClO_4}$ (p.a., Lachema Brno) with redistilled water and standardization against HgO and NaI with a reproducibility better than 0.2% according to the equation $\mathrm{HgO + 4\ NaI + H_2O \rightleftarrows 2\ NaOH + Na_2[HgI_4]}$ and $\mathrm{NaOH + HClO_4 \rightleftarrows NaClO_4 + H_2O}$.

Sodium hydroxide, 1 M, was prepared from the exact weight of pellets (p.a., Aldrich Chemical Company) with carbon-dioxide free redistilled water. The solution was stored for several days in a polyethylene bottle. This solution was standardized against a solution of potassium hydrogenphthalate using the Gran method in the MAGEC program [29] with a reproducibility of 0.1%. Mercuric oxide, sodium iodide, and sodium perchlorate, (p.a. Lachema Brno) were not further purified. Other solutions were prepared from analytical-reagent grade chemicals. Twice-redistilled water was used in the preparation of solutions.

3.2. Apparatus

The free hydrogen-ion concentration h was measured via emf on an OP-208/1 digital voltmeter (Radelkis, Budapest) with a precision of ±0.1 mV with the use of a G202B glass electrode (Radiometer, Copenhagen) and an OP-8303P commercial SCE reference electrode (Radelkis, Budapest). Titrations were performed in a water-jacketed double-walled glass vessel of 100 mL, closed with a Teflon bung containing the electrodes, an argon inlet, a thermometer, a propeller stirrer and a capillary tip from a micro-burette. All emf measurements were carried out at 25.0 ± 0.1 °C. When sulphoazoxines were titrated a stream of argon gas was bubbled through the solution both for stirring and to maintain an inert atmosphere. The argon was passed through an aqueous ionic medium by prior passage through one or two vessels also containing the titrand medium before entering the corresponding titrand solution. The gas is best introduced under the surface of the titrand. Sometimes the flow had to be stopped while emf was measured. The burettes used were syringe micro-burettes of 1250 μL capacity (META, Brno) with a 2.5 cm micrometer screw [30]. The polyethylene capillary tip of the micro-burette was immersed into a solution when adding reagent but pulled out after each addition in order to avoid leakage of reagent during the pH reading. The micro-burette was calibrated by ten replicate determinations of the total volume of delivered water by weighing on a Sartorius 1712 MP8 balance and results were evaluated statistically, leading to a precision of ±0.015% in added volume over the whole volume range.

The pH-spectrophotometric titration was performed outside the cuvette with the use of a SPECORD M400 spectrophotometer (Zeiss, Jena) and was carried out as described in a prior paper [10].

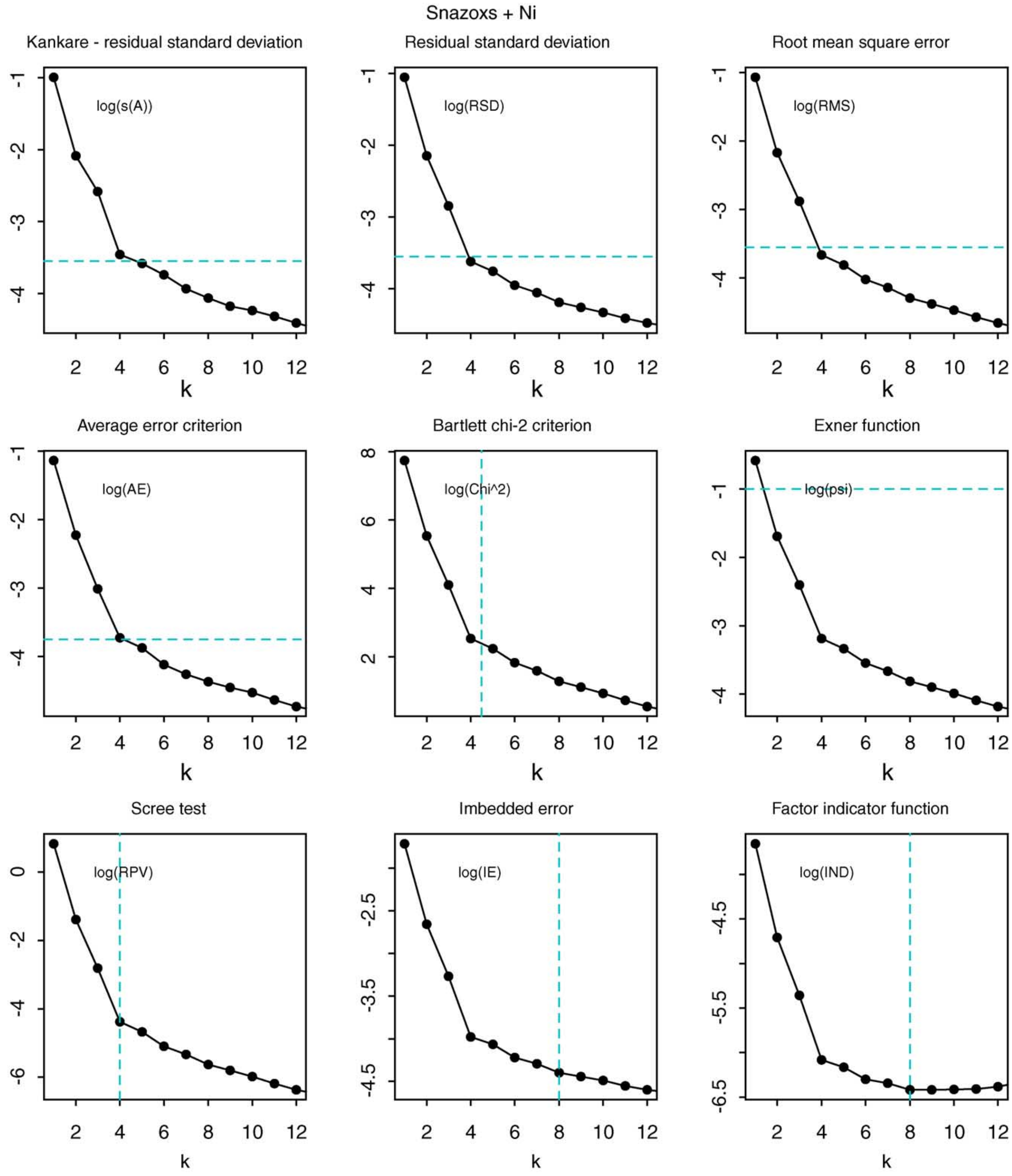


Fig. 1. The logarithmic dependence of nine index methods as a function of the number of principal components *k* for the absorbance matrix of complex-forming equilibria of SNAZOXS with Ni^{2+}: *first row:* Kankare's residual standard deviation $s_k(A)$, Residual standard deviation *RSD*, Root mean square error *RMS*; *second row:* average error criterion *AE*, Bartlett χ^2 criterion, Exner ψ function; *third row:* Scree test *RPV*, imbedded error function *IE*, factor indicator function *IND*.

3.3. Procedure for the determination of the chemical model

(1) *Experimental error of absorbance measurements*, $s_{\mathrm{inst}}(A)$: The INDICES algorithm [18,19] should be used with solutions of potassium dichromate to evaluate $s_{\mathrm{inst}}(A)$, [18]. The graph of $s_{\mathrm{k}}(A) = f(k)$ consists of two straight lines intersecting at $\{s^*_{\mathrm{k}}(A); k^*\}$ where k^* is the matrix rank for the system. Since $k^* = 1$ for $K_2Cr_2O_7$, the value of $s_{\mathrm{k}}(A)$ for $k^* = 1$ is a good estimate of the experimental error, $s_{\mathrm{inst}}(A) = s^*_1(A)$.

(2) *Experimental design*: Since preparation of a large number of separate solutions is tedious, simultaneous monitoring of absorbance and pH during titration is valuable. The total concentrations of the components should be varied between limits as wide as possible, so the mole-ratio methods are useful. In a titration, the total concentration of one of the components changes incrementally over a relatively wide range, but the total concentrations of the other components change only by dilution, or not at all if they are present at the same concentration in the titrant and titrand. However, absorbance cannot be varied over a large range without decreasing the precision of its measurement, and is effectively confined to a range of about one order of magnitude, e.g., $0.1 < A < 1.2$, though the range of concentrations measured can be increased by the use of different path-lengths, e.g., 5, 1 and 0.1 cm. Complexation equilibria of both sulphoazoxines were studied in the visible region, 380–760 nm. The wavelength-range selected was such that every species made a significant contribution to the absorbance. Little information was obtained in regions of great spectral overlap or where the molar absorptivities of two or more species were linearly interdependent because the change in absorbance with changes in c_{M}, c_{L} and c_{H} then became rather small. Where only a small number of wavelengths was used, those of maxima or shoulders were chosen, because small errors in setting the wavelength were then less important. It was best to use wavelengths at which the molar absorptivities of the species differed greatly, or a large number of wavelengths spaced at equal intervals.

(3) *Number of light-absorbing species*: To improve the data, Kankare's method [21] of PCA data regeneration was used for all points with excessive error. A data point was considered to have an excessive error and to be an outlier if the absolute value of the difference between the measured absorbance and the PCA regenerated absorbance was greater than three times the standard deviation; thus data points with large errors were easily spotted and removed. The smoothed data matrix was then PCA analyzed, yielding, hopefully, more reliable results. When no outliers (grossly erroneous points) were present in the spectra examined, $s^*_{\mathrm{k}}(A) \le s_{\mathrm{inst}}(A)$ was valid. The INDICES [18,19] determine the number of dominant species to be present in equilibrium mixture. All spectra evaluation and data simulation were performed in the S-Plus programming environment and the INDICES algorithm [18] is available on the Internet [19]. Most index methods are functions of the number of $PC(k)$s into which the spectral data are usually plotted against k, and when the $PC(k)$ reaches the value of the experimental error of the spectrophotometer used, the corresponding k represents the number of significant components in a mixture, $n_{\mathrm{c}} = k$. The dependence $f(k)$ decreases steeply with the increasing number of PCs as long as the PCs are significant. When k is exhausted the indices fall off, some of indices even displaying a minimum. At this point $n_{\mathrm{c}} = k$ for all indices except the eigenvalue g for which $n_{\mathrm{c}} = k + 1$ is valid. The index values at this point can be predicted from the properties of the noise, which may be used as a criterion to determine n_{c}.

(4) *List of proposed species*: A search should begin with the major species indicated by preliminary data analysis. Suggested species can then be added one at a time. Model selection was based on finding the lowest U value and the best spectra fitting.

(5) *Choice of computational strategy*: The input data should specify whether β_{pqr} or $\log \beta_{pqr}$ values were to be refined, multiple regression (MR) or non-negative linear least-squares (NNLS) were desired, baseline correction was to be performed, etc, [25,26]. In description of the model, it should be indicated whether stability constants were to be

Table 1
Estimated number of light-absorbing species $n_{\mathrm{c}} = k$ and the experimental error, $s_{\mathrm{inst}}(A) = s_{\mathrm{k}}(A)$ by the derivative technique of PCA methods (INDICES program) for the SNAZOXS complex-forming system with six different metals

System	Predicted k and corresponding $s_{\mathrm{k}}(A)$ (mAU) for precise methods and k for approximate methods								
	$s_{\mathrm{k}}(A)$	*RSD*	*RMS*	*AE*	χ^2	*RPV*	ψ	*IE*	*IND*
Cd^{2+}	3, 0.63	3, 0.50	3, 0.48	3, 0.40	3	3	3	3	3
Co^{2+}	4, 0.40	4, 0.32	4, 0.31	4, 0.22	4	4	4	4	4
Cu^{2+}	3, 1.51	3, 1.17	3, 1.12	3, 0.89	3	3	3	3	3
Ni^{2+}	4, 0.31	4, 0.27	4, 0.26	4, 0.23	4	4	4	4	4
Pb^{2+}	3, 0.85	3, 0.83	3, 0.81	3, 0.50	3	3	3	3	3
Zn^{2+}	3, 0.63	3, 0.43	3, 0.40	3, 0.36	3	3	3	3	3

The $n \times m$ absorbance matrix is for n solutions with total concentrations of c_{M}, c_{L} and c_{H}, with molar ratio $q_{\mathrm{M}} = c_{\mathrm{M}}/c_{\mathrm{L}}$ and measured at m wavelengths. Index algorithms used: $s_{\mathrm{k}}(A)$, Kankare's residual standard deviation; *RSD*, residual standard deviation; *RMS*, root mean square error; *AE*, average error criterion; χ^2, Bartlett χ^2 criterion; ψ, Exner ψ function; *RPV*, Scree test; *IE*, imbedded error function; *IND*, factor indicator function.

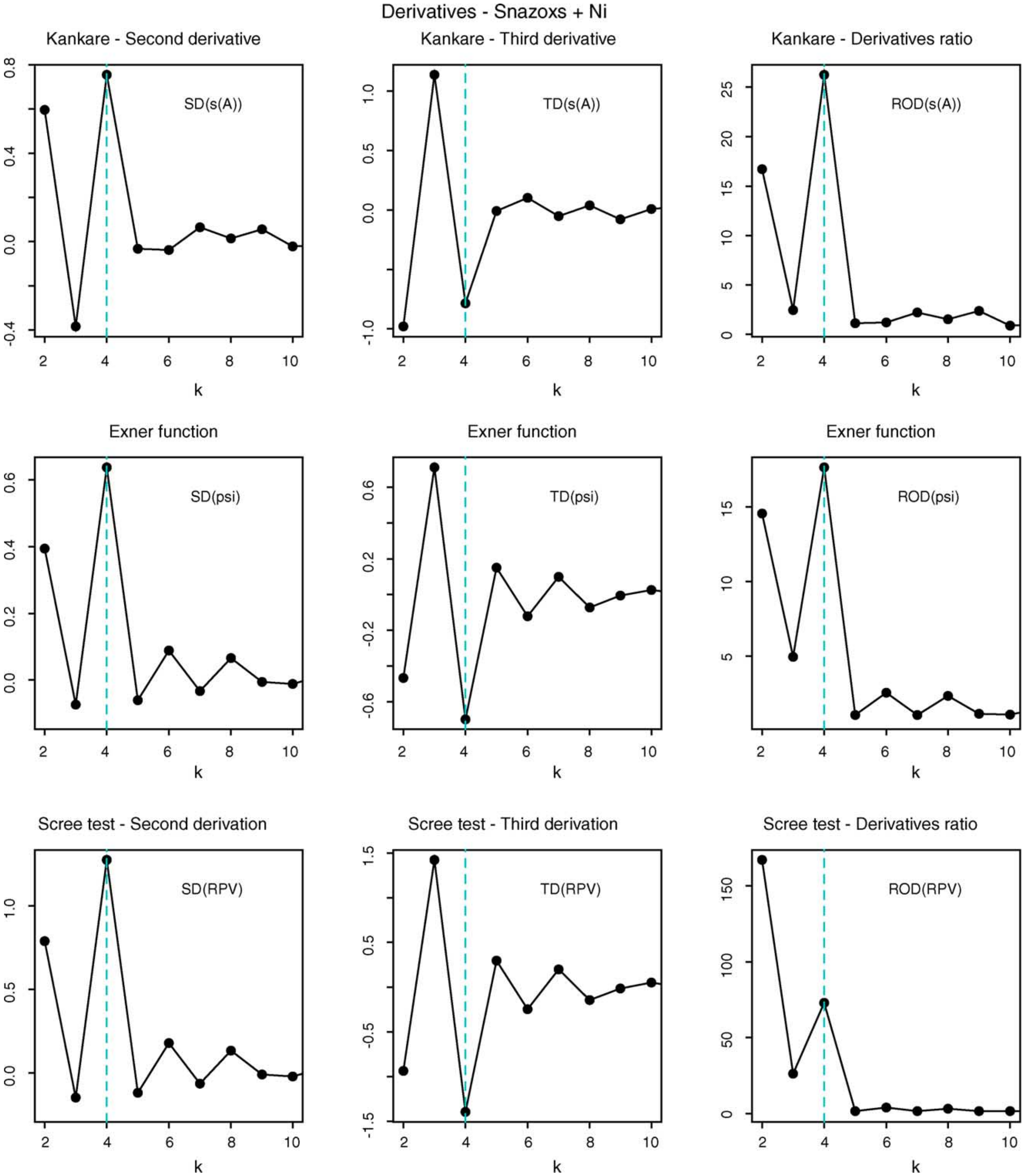


Fig. 2. The derivatives detection criteria of index functions applied for the absorbance data set of complex-forming equilibria from the system of Fig. 1: *first row:* Kankare residual standard deviation $s_k(k)$ the second derivative $SD(s_k(A))$ (left), the third derivative $TD(s_k(A))$ (middle), the derivatives ratio $ROD(s_k(A))$ (right); *second row:* Exner ψ function, the second derivative $SD(\psi)$ (left), the third derivative $SD(\psi)$ (middle), the derivatives ratio $ROD(\psi)$ (right); *third row:* Scree test, the second derivative $SD(RPV)$ (left), the third derivative $SD(RPV)$ (middle), the derivatives ratio $ROD(RPV)$ (right); *fourth row:* Factor indicator function IND, the second derivative $SD(IND)$ (left), the third derivative $SD(IND)$ (middle), the derivatives ratio $ROD(IND)$ (right).

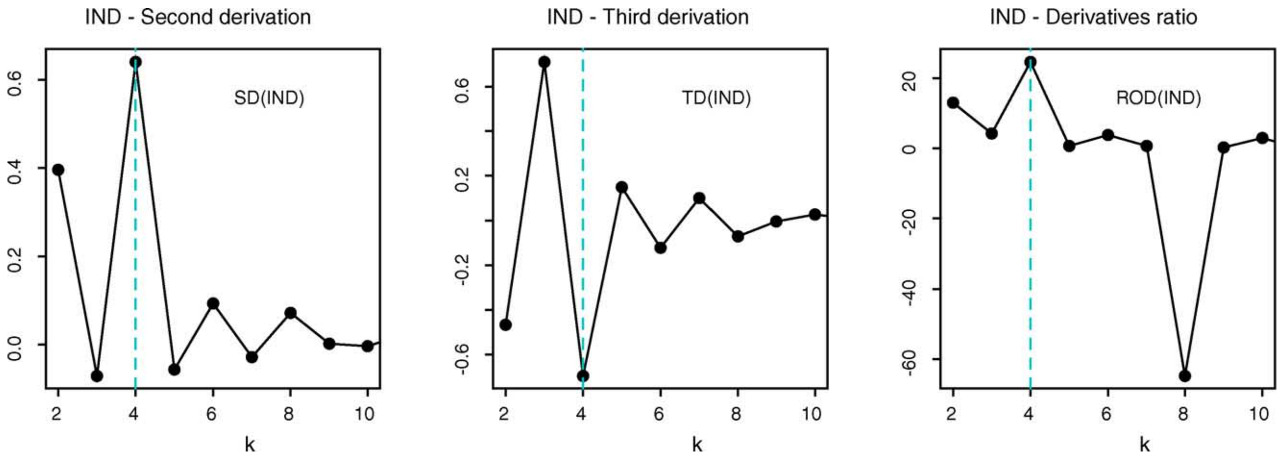


Fig. 2. (*Continued*).

Table 2
The most probable chemical model in complex-forming equilibria of the SNAZOXS + Me^{z+} system by regression spectra analysis using SQUAD(84) with the standard deviations of the estimated stability constant, log $\beta_{pqr}(s(\beta_{pqr}))$ of the last valid digits in brackets

	Cd^{2+}	Co^{2+}	Cu^{2+}	Ni^{2+}	Pb^{2+}	Zn^{2+}
c_L (mol/L)	0.000773	0.000429	0.000429	0.000426	0.000429	0.000429
Range q_M	0.02–2.82	0.05–2.32	0.02–2.29	0.02–1.44	0.02–3.01	0.02–2.13
Range pH	5.14–5.20	4.69–4.78	5.69–4.78	4.72–4.78	4.66–4.78	4.70–4.78
n	17	19	17	20	20	20
m	28	28	28	28	28	28
n_c	3	4	3	4	3	3
$s_k(A)$ (mAU)	0.71	0.26	1.16	0.32	0.66	0.57
Estimated stability constant, log $\beta_{pqr}(s(\beta_{pqr}))$						
ML	4.50(3)	5.75(6)	6.69(6)	6.44(8)	5.63(5)	5.11(3)
ML_2	8.36(7)	9.79(9)	11.40(7)	10.91(11)	9.97(9)	8.84(5)
ML_3	–	13.05(2)	–	15.07(10)	–	–
The degree-of-fit test by the statistical analysis of residuals						
$\bar{e}$ (mAU)	0	6.19000e − 12	3.11000e − 12	4.30000e − 12	5.19000e − 12	−8.4500e − 13
$\lvert\bar{e}\rvert$ (mAU)	2.1	0.77	1.55	0.95	1.67	1.05
$s(e)$ (mAU)	3.2	1.08	2.54	1.39	2.73	1.58
$g_1(e)$ (≈0)	0.093	−0.2	0.84	0.04	0.83	0.58
$g_2(e)$ (≈3)	4.04	2.26	5.18	2.87	4.96	4.27
R (%) (<0.5)	0.495	0.143	0.34	0.184	0.408	0.216

The parameter reliability is proven with degree-of-fit statistics such as the mean of residuals $\bar{e}$ (mAU), the mean residual $|\bar{e}|$ (mAU), the standard deviation of residual $s(e)$ (mAU) which is equal to the standard deviation of absorbance after termination of the regression process, $s(A)$ (mAU), the residual skewness $g_1(e)$ and residual kurtosis $g_2(e)$ and the Hamilton *R-factor* (%). Experimental conditions: c_L (SNAZOXS), $I = 0.1$ ($NaClO_4 + Na_3PO_4 + HClO_4$), 25 °C, path length 1.000 cm, $s_{inst}(A) = 0.45$ mAU (Specord M40, Zeiss, Jena Germany).

Table 3
Number of light-absorbing species $n_c = k$ and the experimental error, $s_{inst}(A) = s_k(A)$ predicted by various PCA methods of the INDICES program for the Naphtylazoxine 6S complex-forming system with six various metals

k and corresponding $s_k(A)$ (mAU) for various index methods								
Metal	$s_k(A)$	*RSD*	*RMS*	*AE*	χ^2	*RPV*	ψ	*IE*	*IND*
Cd^{2+}	4, 0.47	4, 0.25	4, 0.23	4, 0.20	4	4	4	4	4
Cu^{2+}	3, 1.41	3, 1.20	3, 1.07	3, 0.91	3	3	3	3	3
Ni^{2+}	4, 1.05	4, 0.89	4, 0.74	4, 0.71	4	4	4	4	4
Zn^{2+}	3, 1.17	3, 0.93	3, 0.83	3, 0.63	3	3	3	3	3

The $n \times m$ absorbance matrix is for n solutions with total concentrations of c_M, c_L and c_H, with molar ratio $q_M = c_M/c_L$ and measured at m wavelengths. Index algorithms used: $s_k(A)$, Kankare's residual standard deviation; *RSD*, residual standard deviation; *RMS*, root mean square error; *AE*, average error criterion; χ^2, Bartlett χ^2 criterion; ψ, Exner ψ function; *RPV*, Scree test; *IE*, imbedded error function; *IND*, factor indicator function.

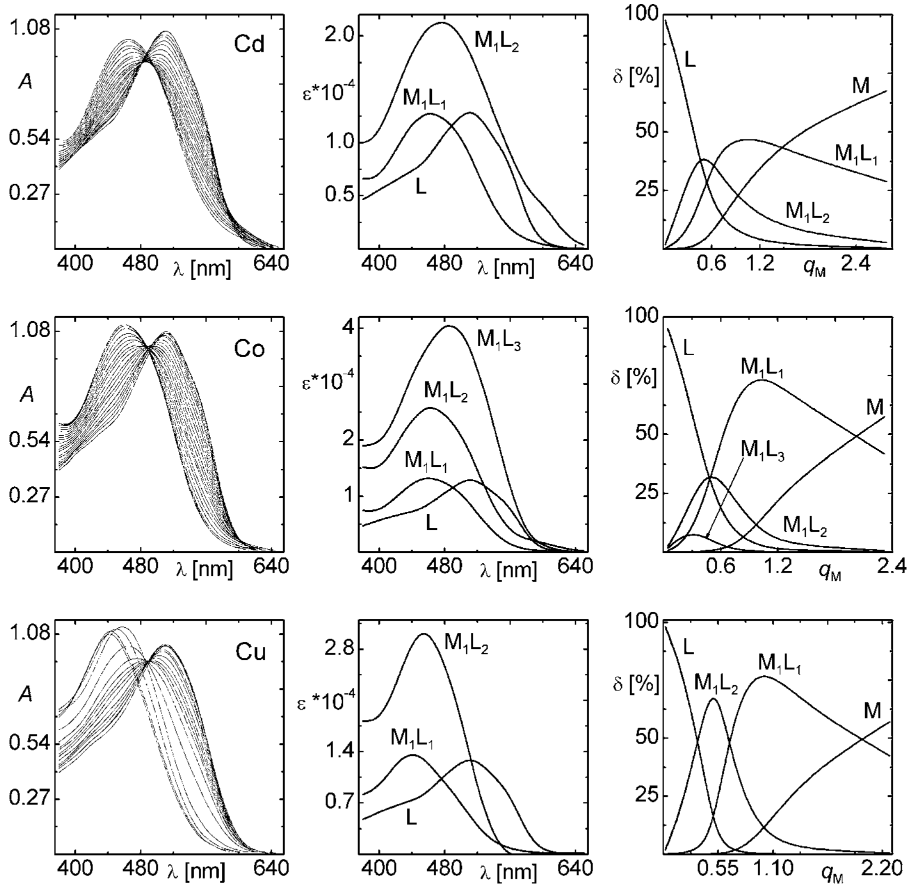


Fig. 3. Complex-forming equilibria of the SNAZOXS systems with three metals presented on the absorption spectra at 25 °C (left), the spectra of molar absorptivities vs. wavelengths for all complex species (middle) and the distribution diagram of the relative concentrations of all of complex species in equilibrium: *first row:* SNAZOXS + Cd^{2+}; *second row:* SNAZOXS + Co^{2+}; *third row:* SNAZOXS + Cu^{2+}.

refined or held constant and whether molar absorptivities were to be refined, and complexes for the species selector should be listed.

(6) *Simultaneous estimation of stoichiometry and stability constants*: A group of complexes in a given equilibrium system was divided into "certain" complexes of known stoichiometry, with stability constants estimated by the trial-and-error method, and "uncertain" complexes, for which the stoichiometry and stability constants were estimated simultaneously by regression analysis. Chemical experience and tables of stability constants helped in making initial guesses as to unknown parameters.

(7) *Diagnostics indicating a correct chemical model*: When a minimization process terminates, some diagnostics are examined to determine whether the results should be accepted. An incorrect chemical model with false stoichiometric indices p, q and r might lead to slow convergence, cyclization or divergence of the minimization. To reach a better chemical model, the following diagnostics should be considered:

First diagnostic – the physical meaning of the parametric estimates: The physical meaning of the stability constants, molar absorptivities, and stoichiometric indices was examined: β_{pqr} and ε_{pqr} should be neither too high nor too low, and ε_{pqr} should not be negative; p, q, r should be very close to integers.

Second diagnostic – the physical meaning of the species concentrations: The calculated distribution of the free concentration of the components and the complexes of the chemical model should reach molarities down to about 10^{-8} M. Since a complex species present at about 1% relative concentration or less in an equilibrium behaves as numerical noise in regression analysis, a distribution diagram makes it easier to quickly judge the contributions of individual species to the total concentration. Since the molar absorptivities will generally be in the range 10^3–10^5 l mole^{-1} cm^{-1}, species present at less than ca. 0.1% relative concentration will affect the absorbance significantly only if their ε is extremely high.

Third diagnostic – standard deviations of parameters: The absolute values of $s(\beta_j)$, $s(\varepsilon_j)$, $s(p_j)$, $s(q_j)$, $s(r_j)$, yield information about the last U-countour of the hyperparaboloid neighbourhood of the pit, $U_{\min}$. For well-conditioned parameters, the last U-contour was a regular ellipsoid, and the standard deviations were reasonably low. High s values were found

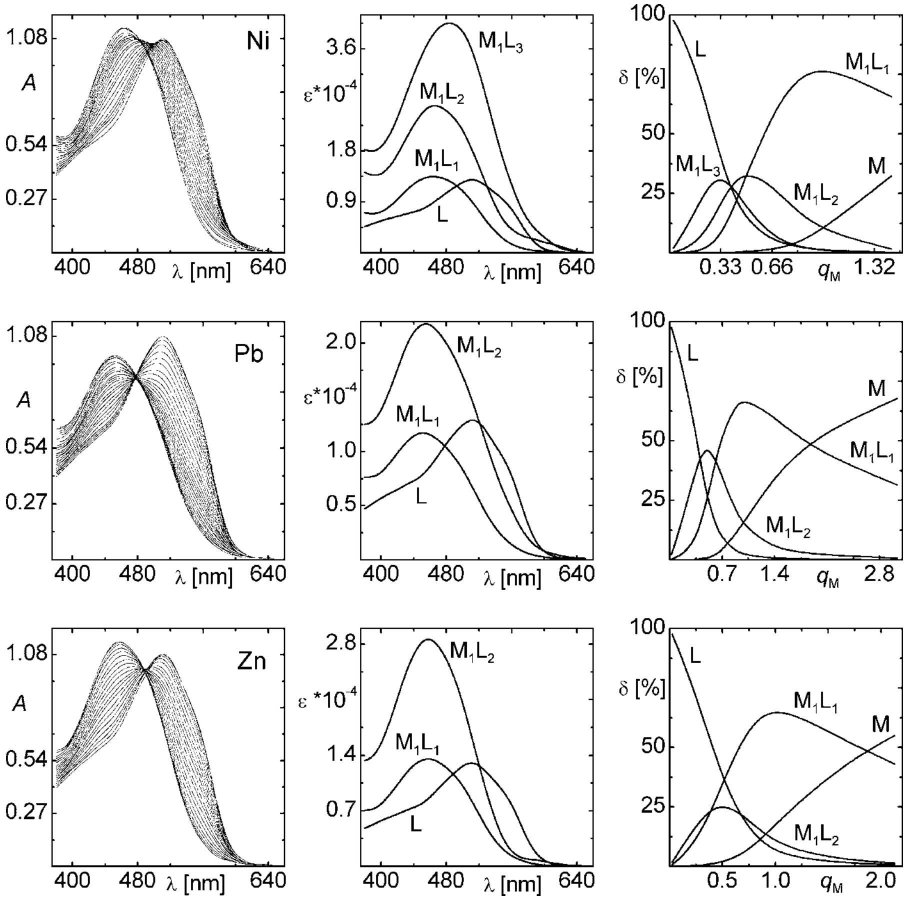


Fig. 4. Complex-forming equilibria of the SNAZOXS systems with three metals presented on the absorption spectra at 25 °C (left), the spectra of molar absorptivities vs. wavelengths for all complex species (middle) and the distribution diagram of the relative concentrations of all of complex species in equilibrium: *first row:* SNAZOXS + Ni^{2+}; *second row:* SNAZOXS + Pb^{2+}; *third row:* SNAZOXS + Zn^{2+}.

with ill-conditioned parameters and a "saucer"-shaped pit. The F_σ test, $s(\beta_j) \times F_\sigma < \beta_j$ should be met. The set of standard deviations of ε_{pqr} for various wavelengths, $s(\varepsilon_{pqr}) = f(\lambda)$ should have a Gaussian distribution; otherwise erroneous estimates of ε_{pqr} are obtained.

Fourth diagnostic – goodness-of-fit test: This diagnostic contains the criteria for testing the correctness of a hypothesis of a chemical model. To identify the "best" true chemical model when several are possible or proposed, and to establish whether or not the chemical model represents the data adequately, the residuals e should be analysed. The goodness-of-fit achieved was easily seen by examination of the differences between the experimental and calculated value of absorbance, $e_i = A_{\mathrm{exp},i,j} - A_{\mathrm{calc},i,j}$. Examination of the spectra and the graph of the predicted absorbance response-surface through all the experimental points should reveal whether the results calculated were consistent and whether any gross experimental errors were made in measurement of the spectra. One of the most important statistics calculated was the standard deviation of the absorbance, $s(A)$, calculated from the set of refined parameters at the termination of minimization process. It was usually compared with the standard deviation of absorbance calculated by PCA in the INDICES program [18,19], $s_k(A)$, and if $s(A) \leq s_k(A)$, or $s(A) \leq s_{\mathrm{inst}}(A)$, the experimental error of the spectrophotometer used, the fit was considered to be good and statistically acceptable. Although statistical analysis of residuals [27] gave the most rigorous test of the degree-of-fit, realistic empirical limits were used. For example, when $s_{\mathrm{inst}}(A) \leq s(A) \leq 0.005$, the goodness-of-fit was taken as acceptable, whereas $s(A) > 0.010$ indicated that a good fit had still not been obtained. The statistical tools applied to a set of residuals have been described previously [10,26,27]. Alternatively, the statistical measures of residuals e can be calculated: the residual mean $\bar{e}$ (known as the bias) should be a value close to zero; the mean residual $|\bar{e}|$ and the residual standard deviation $s(e)$ should be close to the absorbance standard deviation $s_{\mathrm{inst}}(A)$; the skewness $g_1(e)$ should be close to zero for a symmetric distribution; the kurtosis $g_2(e)$ should be close to 3 for a Gaussian distribution; if the Hamilton *R-factor* of relative fit, expressed as a percentage ($R \times 100\%$), was <0.5% then the fit was taken as an excellent one, but if it was >2% the fit was taken to be poor. The *R-factor* gave a rigorous test of the null hypothesis H_0 (giving R_0) against the alternative H_1 (giving R_1). H_1 could be rejected at the α significance level if $R_1/R_0 > R_{(k,n-k,\alpha)}$, where n was the number

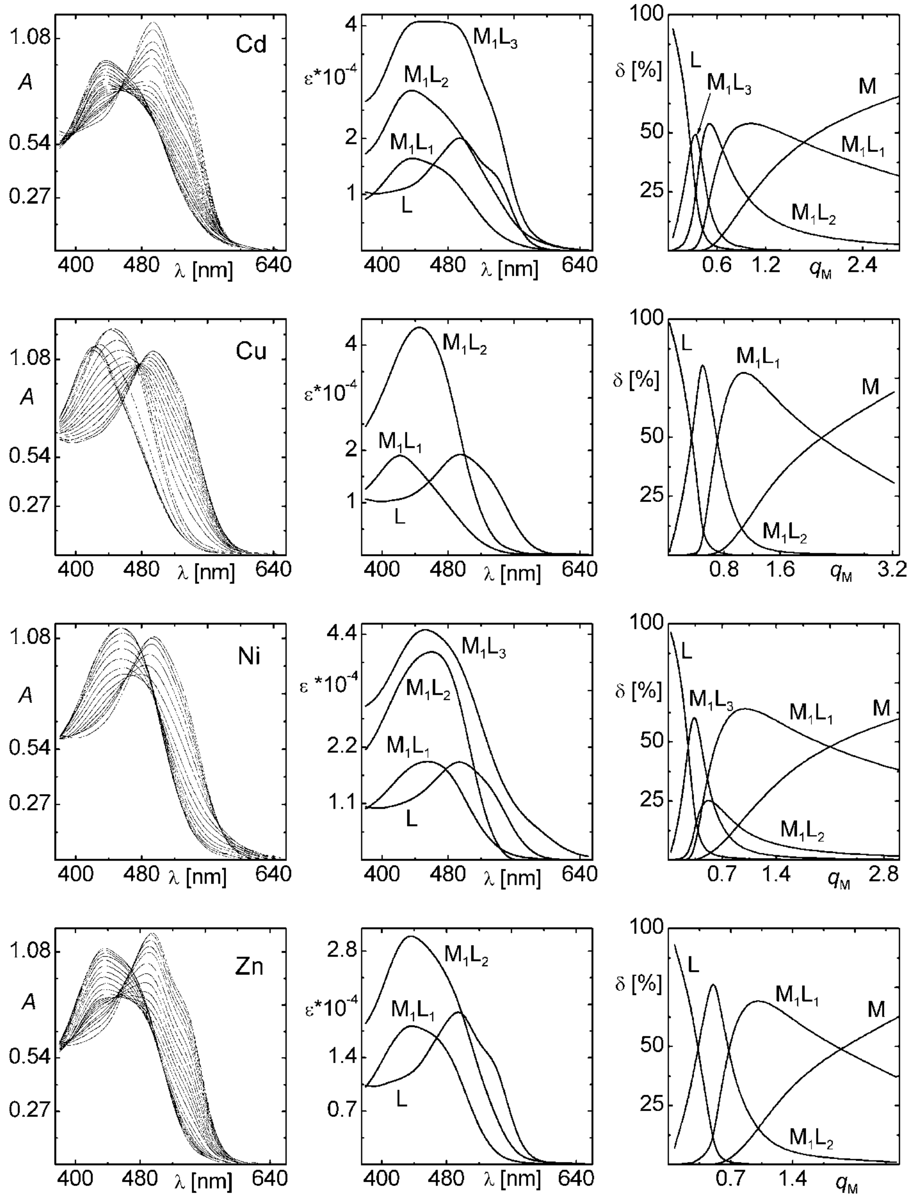


Fig. 5. Complex-forming equilibria of the Naphtylazoxine 6S systems with four metals is presented on the absorption spectra at 25 °C (left), the spectra of molar absorptivities vs. wavelengths for all complex species (middle) and the distribution diagram of the relative concentrations of all of complex species in equilibrium: *first row:* Naphtylazoxine 6S + Cd^{2+}; *second row:* Naphtylazoxine 6S + Cu^{2+}; *third row:* Naphtylazoxine 6S + Ni^{2+}; *fourth row:* Naphtylazoxine 6S + Zn^{2+}.

of experimental points, k was the number of unknown parameters, and $(n - k)$ was the number of degrees of freedom. The value of $R_{(k,n-k,\alpha)}$ might be found in statistical tables.

Fifth diagnostic – deconvolution of spectra: resolution of each experimental spectrum into spectra for the individual species proved whether the experimental design was efficient. If for a particular concentration range the spectrum consisted of just a single component, further spectra for that range would be redundant, though they should improve the precision. In ranges where many components contribute significantly to the spectrum, several spectra should be measured.

4. Results and discussion

4.1. The number of complex species

Spectral data may be subject to random errors which cannot be eliminated from the data, and to systematic errors which are sometimes difficult to identify and eliminate. The sources of systematic error in pH measurement are documented [29]. Coloured impurities in sulphoazoxines may have an acid-base character in which case the background colour will vary with pH. Some indicators may separate from solution and/or be adsorbed on the cuvette walls, and at higher concentrations oligomers and micelles may be formed.

Table 4
The most probable chemical model in the Naphtylazoxine 6S complex-forming system + Me^{z+} by regression spectra analysis using SQUAD(84) with the standard deviations of the estimated stability constant, log $\beta_{pqr}(s(\beta_{pqr}))$ in the last valid digits in brackets

	Cd^{2+}	Cu^{2+}	Ni^{2+}	Zn^{2+}
c_L (mol/L)	0.000301	0.000303	0.000297	0.000307
Range q_M	0.05–3.45	0.04–3.24	0.03–5.42	0.06–2.56
Range pH	4.72–4.78	4.69–4.78	4.64–4.78	4.7–4.78
n	15	13	14	14
m	28	28	28	28
n_c	4	3	4	3
$s_k(A)$ (mAU)	0.25	0.78	0.86	0.74
Estimated stability constant, log $\beta_{pqr}(s(\beta_{pqr}))$				
ML	6.08(4)	7.80(8)	6.35(12)	7.01(8)
ML_2	11.44(7)	13.41(14)	11.43(19)	12.65(15)
ML_3	16.06(11)	–	16.68(24)	–
The degree-of-fit test by the statistical analysis of residuals				
$\bar{e}$ (mAU)	0	0	0	0
$\lvert\bar{e}\rvert$ (mAU)	0.54	2.59	2.66	2.34
$s(e)$ (mAU)	0.9	4.67	4.29	4
$g_1(e)$ (≈0)	0.28	0.89	−0.05	0.41
$g_2(e)$ (≈3)	3.72	4.02	2.57	4.26
R (%) (<0.5)	0.13	0.602	0.556	0.56

The parameter reliability is proven with degree-of-fit statistics such as the mean of residuals $\bar{e}$ (mAU), the mean residual $|\bar{e}|$ (mAU), the standard deviation of residual $s(e)$ (mAU) which is equal to the standard deviation of absorbance after termination of the regression process, $s(A)$ (mAU), the residual skewness $g_1(e)$ and residual kurtosis $g_2(e)$ and the Hamilton *R-factor* (%). Experimental conditions: c_L (SNAZOXS), $I = 0.1$ ($NaClO_4 + Na_3PO_4 + HClO_4$), 25 °C, path length 1.000 cm, $s_{inst}(A) = 0.45$ mAU (Specord M40, Zeiss, Jena Germany).

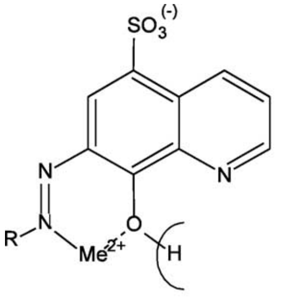
Changes in concentrations cause a systematic rather than random error. Determination of the number of light-absorbing components in an equilibrium mixture seems to be an important point before the formulation of the hypothesis of a chemical model. When the absorbance matrix monitoring the complex-forming equilibria of SNAZOXS (or Naphtylazoxine 6S) with various metal cations was subjected to PCA indices analysis using covariance about the origin, nine characteristics $s_{inst}(A)$, *RSD*, *RMS*, *AE*, χ^2, ψ, *RPV*, *IE* and *IND* resulted. The break on the curve $PC(k) = f(k)$ may be used as a guide to selecting the primary eigenvalues. Deducing the exact size of the true component space is usually a difficult problem because of experimental error. The various techniques developed to solve this problem can be divided into precise methods ($s_{inst}(A)$, *RSD*, *RMS* and *AE* based on a knowledge of the experimental error of absorbance of spectrophotometer used $s_{inst}(A)$) and approximate methods (χ^2, ψ, *RPV*, *IE* and *IND*) requiring no knowledge of this experimental error. Obviously, methods in the first category are preferred when the error is known. The number of light-absorbing components n_c can be estimated from indices by comparing them with the experimental error, using the noise level $s_{inst}(A)$, RSD_{inst}, RMS_{inst} and AE_{inst} as a threshold. This is the common criterion to determine n_c for precise methods. The point where $k = n_c$ from dependence of the indices on the number of principal components k to calculate them is then proposed for use. Comparing, for example, an index value, *RSD* calculated for various index k to the estimated experimental error: if the *RSD* is greater than the estimated error RSD_{inst}, the number of factors k is insufficient. If the *RSD* is approximately equal to the estimated error RSD_{inst}, the proper number of factors k is appropriate. The dependence $PC(k) = f(k)$ decreases steeply with the increasing index k as long as the k's are significant, i. e. one finds the point where the slope of the indicator function $PC(k) = f(k)$ changes (Fig. 1): for the absorbance matrix of the SNAZOXS-Ni^{2+} complex-forming system the $s_k(A)$, *RSD*, *RMS* and *AE* indices lead to $n_c = 4$. However, information about the experimental error is often lacking and approximate methods in the second category must be employed because they offer a solution, albeit of a more dubious nature: in the case of the Exner ψ function the most suitable criterion seems to be to indicate the break on the curve $\psi(k) = f(k)$. For $k = 4$ the break appears proving that four species are in equilibrium mixture. For the Scree test $RPV(k) = f(k)$, the residual percentage variance $RPV(k)$ plotted against the number of components k shows that the curve drops rapidly and levels off at $n_c = 4$. This point can be used to reduce the components space. On Fig. 1, we see that no minimum appears in the $IE(k) = f(k)$ function. Unfortunately, in this case the $IE(k) = f(k)$ function exhibits the minimum at $k = 8$ which is definitely false. However, the *IE* shows a progressive decrease on going from $k = 1$ to $k = 4$ with a break at $k = 4$. This perhaps hints that four species are responsible for the absorbance matrix. The $IND(k) = f(k)$ function, similar to the $IE(k) = f(k)$ function, reaches a minimum at $k = 8$ which is more pronounced and occurs in situations where $IE(k) = f(k)$ exhibits no clear minimum. Unfortunately, the indicated number of species $k = 8$ is, as with the *IE* function, quite false. Similarly as for the $IE(k) = f(k)$ function the break on this curve appears at $k = 4$ and therefore this k can be taken as evidence that four species are responsible for the absorbance matrix. Table 1 shows results for the SNAZOXS complex-forming system with six various metals when the absorbance matrix was measured for n solutions with total concentrations of c_M, c_L and c_H and molar ratio $q_M = c_M/c_L$ at m wavelengths and with the use of the nine index algorithms, $s_k(A)$, *RSD*, *RMS*, *AE*, χ^2, ψ, *RPV*, *IE* and *IND*. The number of complex species in the mixture was evaluated more reliably with the use of the second derivatives *SD*, the third derivatives *TD* and the derivatives ratio *ROD* and using the following rules (Fig. 2): (a) The curve of the second derivative of the index function $SD(PC(k)) = f(k)$ exhibits a maximum for the true index $k = n_c$. (b) The curve of the third derivative of the index function $TD(PC(k)) = f(k)$ crosses zero and reaches a negative minimum which can be used as a criterion for the best estimate of index k being equal to n_c, $k = n_c$ (c) The curve of the ratio of derivatives $ROD(PC(k)) = f(k)$ should reach a first maximum at the point where $k = n_c$. Tables 1 and 3 show the predicted number of complex species of the two SNAZOXS and Naphtylazoxine 6S complex-forming systems, with various metal cations using the methods demonstrated in Fig. 2 (Tables 2 and 3, Figs. 3–5).

4.2. Complexation equilibria of SNAZOXS and Naphtylazoxine 6S

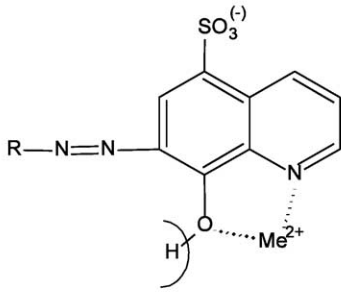
Applying the procedure of efficient experimentation and computational strategy on the mole ratio spectra, the ratio $q_M = c_M/c_L$ is varied by keeping c_L constant and varying c_M. The metalochromic indicator SNAZOXS forms yellow complexes with cadmium(II), cobalt(II), copper(II), lead(II), nickel(II) and zinc(II) at pH 5. In solutions with an excess of the ligand, $q_M < 0.5$, the complex ML_3 usually predominates while in equimolar solutions ML_2 and ML complexes exist. The sulphoazoxine Naphtylazoxine 6S forms yellow complexes only with cadmium(II), copper(II), nickel(II) and zinc(II) ions. Table 2 presents the results of the chemical model determination for the SNAZOXS system and Table 4 for the Naphtylazoxine 6S system. The *chemical model* concerns a guess as to the number of light-absorbing species coexisting in the equilibrium mixture, their stoichiometry, their stability constants which are estimated by regression analysis and at the same time the curves of molar absorption coefficients in dependence on wavelength. For a set of current values of β_{pqr} the free concentrations of metal [M], ligand [L] followed by the concentrations of all the complexes in equilibrium mixture, $[M_pL_qH_r]_j$, $j = 1,\ldots, n_c$, forming for n solutions the matrix $\boldsymbol{C}$.

In addition to the fit achieved, it is also necessary to examine the physico-chemical sense of the model parameters estimates, such as positive values in the concentration matrix, positive values of molar absorptivities, and also the concentration fraction of the complex species in the mixture. If a complex species is present in a fraction lower than 5–10%, the evaluation of such an equilibrium can fail: from the spectral point of view it acts as a “noise” only, insufficient for an evaluation of its own equilibrium and complicating the evaluation of other equilibria. This problem can be solved by augmenting the set of spectra with a single spectrum of the molar absorptivities of the unknown species. In searching for a chemical model the hypothesis of three species L, ML and ML_2 was tested in the first step. In accordance with results of the PCA indices analysis the hypothesis of four species was also tested, and better fitness through spectra points was obtained. Therefore, the final chemical model contained four light-absorbing components in most systems.

The central metal ion is bonded into the complex on one side to the oxygen atom of the phenolic hydroxy group at the quinoline ring; the chelate ring closure is accomplished by bonding either to the heterocycle nitrogen or to one of the nitrogen atoms of the azo group, probably the more remote one. The structure of the various complexes formed can thus be represented by the formulas I and II.



I



II

Cherkesov [31] explained the formation of a covalent bond of the central metal ion to the azo group nitrogen, hence the formation of complex structure I. In this case the six-membered chelate ring is completed by the bond of the central ion to the azo group nitrogen more remote from the quinoline ring. Type I complexes are protonized at the heterocycle nitrogen, their existence thus explaining the occurrence of protonized complexes. As the pH of solutions increases the bond of the central ion to the azo group nitrogen is disrupted and a bond of the ion to the heterocycle nitrogen of the quinoline system is formed; hence complex structure II appears [32].

5. Conclusion

1. Generally, the most reliable index methods seem to be those based on a knowledge of experimental error of ab-

sorbance. The Wernimont–Kankare procedure is a reliable method for the determination of the instrumental standard deviation of the spectrophotometer used.

2. Index methods are all based on finding the point where the slope of the index function changes, and therefore derivates application is very useful. Due to the large variations in the index values even at a logarithmic scale they do not reach an obvious point where the slope changes. For more than three components in the mixture, the modification of Elbergali et al. with the second or third derivative and derivative ratio function seem to be an useful resolution tool enabling the correct prediction of the number of components in spectra for all index functions.
3. Precise methods based on a knowledge of the instrumental error $s_{\mathrm{inst}}(A)$ are preferred as the more reliable procedure as they always predict the correct number of components and even the presence of minor species in mixture.
4. A *chemical model* (i. e. the number of complexes, their stoichiometry, their stability constants and their curves of molar absorption coefficients with a distribution diagram of relative concentrations of all species) of the following systems was found at $I \approx 0.1$ and 25 °C. The standard deviations of the estimated stability constant, $\log \beta_{pqr}(s(\beta_{pqr}))$ of the last valid digits in brackets are in brackets:

SNAZOXS + Cd^{2+}: for q_{M} 0–2.8 it lead to 3 light-absorbing species L, ML, ML_2 with estimates of stability constants $\log \beta_{110} = 4.50(3)$, $\log \beta_{120} = 8.36(7)$.
SNAZOXS + Co^{2+}: for q_{M} 0–2.3 it lead to 4 light-absorbing species L, ML, ML_2, ML_3 with $\log \beta_{110} = 5.75(6)$, $\log \beta_{120} = 9.79(9)$, $\log \beta_{130} = 13.05(2)$.
SNAZOXS + Cu^{2+}: for q_{M} 0–2.3 it lead to 3 light-absorbing species L, ML, ML_2 with $\log \beta_{110} = 6.69(6)$, $\log \beta_{120} = 11.40(7)$.
SNAZOXS + Ni^{2+}: for q_{M} 0–1.5 it lead to 4 light-absorbing species L, ML, ML_2, ML_3 with $\log \beta_{110} = 6.44(8)$, $\log \beta_{120} = 10.91(11)$, $\log \beta_{130} = 15.07(10)$.
SNAZOXS + Pb^{2+}: for q_{M} 0–3.01 it lead to 3 light-absorbing species L, ML, ML_2 with $\log \beta_{110} = 5.63(5)$, $\log \beta_{120} = 9.97(9)$.
SNAZOXS + Zn^{2+}: for q_{M} 0–2.1 it lead to 3 light-absorbing species L, ML, ML_2 with $\log \beta_{110} = 5.11(3)$, $\log \beta_{120} = 8.84(5)$.
NAFTYLAZOXIN + Cd^{2+}: for q_{M} 0–3.5 it lead to 4 light-absorbing species L, ML, ML_2, ML_3 with $\log \beta_{110} = 6.08(4)$, $\log \beta_{120} = 11.44(7)$, $\log \beta_{130} = 16.06(11)$.
NAFTYLAZOXIN + Cu^{2+}: for q_{M} 0–3.2 it lead to 3 light-absorbing species L, ML, ML_2 with $\log \beta_{110} = 7.80(8)$, $\log \beta_{120} = 13.41(14)$.
NAFTYLAZOXIN + Ni^{2+}: for q_{M} 0–5.4 it lead to 4 light-absorbing species L, ML, ML_2, ML_3 with $\log \beta_{110} = 6.35(12)$, $\log \beta_{120} = 11.43(19)$, $\log \beta_{130} = 16.68(24)$.
NAFTYLAZOXIN + Zn^{2+}: for q_{M} 0–2.6 it lead to 3 light-absorbing species L, ML, ML_2 with $\log \beta_{110} = 7.01(8)$, $\log \beta_{120} = 12.65(15)$.

Acknowledgements

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