

# Number of species in complexation equilibria of *o*-, *m*- and *p*-CAPAZOXS with Cd<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Pb<sup>2+</sup> and Zn<sup>2+</sup> ions by PCA of UV–vis spectra

Milan Meloun\*, Tomáš Syrový

Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, CZ-532 10 Pardubice, Czech Republic

Received 1 March 2005; received in revised form 23 February 2006; accepted 15 March 2006

Available online 9 May 2006

## Abstract

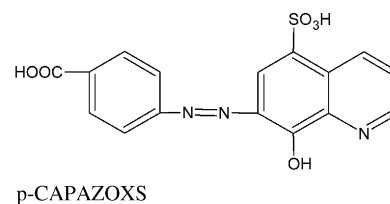
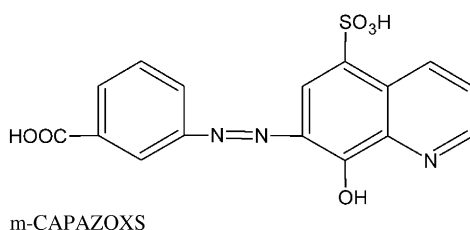
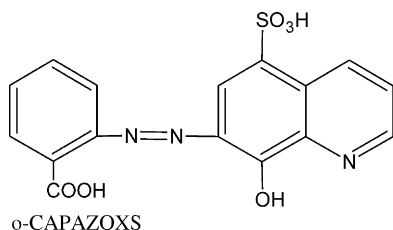
A critical comparison of the selected derivative principal component analysis (PCA) methods on the absorbance matrix data concerning the complexation equilibria between *o*-CAPAZOXS and Cd<sup>2+</sup>, Pb<sup>2+</sup> and Zn<sup>2+</sup> or *m*-CAPAZOXS and Cd<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup> and Zn<sup>2+</sup> or *p*-CAPAZOXS and Cd<sup>2+</sup> and Zn<sup>2+</sup> at 25 °C is provided. As the number of complex species in a complex-forming equilibria mixture is an important step in spectral data treatment, the nine selected index functions for the prediction of the number of light-absorbing species that contribute to a set of spectra is critically tested by the PCA. An improved identification with the second SD(AE) or third derivative TD(AE) and derivative ratio function ROD(AE) for the average error criterion AE is preferred. After the number of various complexes formed the stability constants of species ML, ML<sub>2</sub> (and ML<sub>3</sub>, respectively) type  $\log \beta_{11}$ ,  $\log \beta_{12}$  (and  $\log \beta_{13}$ , respectively) for the system of *o*-CAPAZOXS (ligand L) with the metals (the standard deviation  $s(\log \beta_{pq})$  of the last valid digits is given in brackets) Cd<sup>2+</sup> (6.39(5) and 11.51(9)), Pb<sup>2+</sup> (4.24(2) and 9.01(2)) and Zn<sup>2+</sup> (5.18(7) and 9.06(10)) and for the system of *m*-CAPAZOXS with Cd<sup>2+</sup> (6.59(20) and 11.51(32)), Co<sup>2+</sup> (7.19(6) and 12.19(8)), Ni<sup>2+</sup> (7.64(7) and 13.39(12)) and Zn<sup>2+</sup> (4.83(3) and 9.57(3)) and for the system of *p*-CAPAZOXS with Cd<sup>2+</sup> (6.44(5), 10.99(10) and 14.57(25)) and Zn<sup>2+</sup> (6.84(16), 13.05(29) and 18.74(43)) at 25 °C are estimated using SQUAD(84) nonlinear regression of the mole-ratio spectrophotometric data. The computational strategy is presented with goodness-of-fit tests and various regression diagnostics capable of proving the reliability of the chemical model proposed.

© 2006 Elsevier B.V. All rights reserved.

**Keywords:** Principal component analysis (PCA); Factor analysis (FA); Rank of matrix; Number of species; Number of components; Spectrophotometer error

## 1. Introduction

The protonation and complex-forming equilibria of some sulphoazoxine oligomers have been studied systematically in the authors laboratory [1]. The protonation constants and the number of oligomer species for five 7-arylazo derivatives of 8-hydroxyquinoline-5-sulphonic acid, sometimes differing only in the steric arrangement of their molecules, viz. SNAZOXS and naphthylazoxine 6S [1] or *o*-, *m*-, *p*-CAPAZOXS, were studied.



The five substances are frequently applied as metalochromic indicators in analytical practice, as they form complex compo-

unds, usually yellow in color, with a series of metals. Analysis of solution equilibria 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

\* Corresponding author. Tel.: +420 466037026; fax: +420 466037068.  
E-mail address: milan.meloun@upce.cz (M. Meloun).

Table 1  
Estimated number of light-absorbing species  $n_c = k$  and the experimental error,  $s_{\text{inst}}(A) = s_k(A)$  by the derivative technique of PCA methods (INDICES program) for the *o*-CAPAZOXS (three metals), *m*-CAPAZOXS (four metals) and *p*-CAPAZOXS (two metals) complex-forming systems with different metals

| System             | $s_k(A)$ | R.S.D.  | RMS     | AE      | $\chi^2$ | RPV | $\psi$ | IE | IND |
|--------------------|----------|---------|---------|---------|----------|-----|--------|----|-----|
| <i>o</i> -CAPAZOXS |          |         |         |         |          |     |        |    |     |
| Cd <sup>2+</sup>   | 3, 0.63  | 3, 0.56 | 3, 0.50 | 3, 0.50 | 5        | 3   | 1      | 8  | 8   |
| Pb <sup>2+</sup>   | 3, 0.56  | 3, 0.49 | 3, 0.40 | 3, 0.40 | 3        | 3   | 1      | 6  | 6   |
| Zn <sup>2+</sup>   | 3, 0.50  | 3, 0.46 | 3, 0.40 | 3, 0.40 | 3        | 3   | 1      | 5  | 5   |
| <i>m</i> -CAPAZOXS |          |         |         |         |          |     |        |    |     |
| Cd <sup>2+</sup>   | 3, 0.63  | 3, 0.20 | 3, 0.20 | 0.18    | 3        | 3   | 1      | 5  | 5   |
| Co <sup>2+</sup>   | 3, 0.50  | 3, 0.20 | 3, 0.20 | 0.16    | 3        | 3   | 1      | 5  | 5   |
| Ni <sup>2+</sup>   | 3, 0.71  | 3, 0.32 | 3, 0.28 | 0.25    | 3        | 3   | 1      | 6  | 6   |
| Zn <sup>2+</sup>   | 3, 0.63  | 3, 0.20 | 3, 0.18 | 0.16    | 3        | 3   | 1      | 8  | 5   |
| <i>p</i> -CAPAZOXS |          |         |         |         |          |     |        |    |     |
| Cd <sup>2+</sup>   | 4, 0.32  | 4, 0.18 | 4, 0.16 | 4, 0.11 | 4        | 4   | 1      | 6  | 6   |
| Zn <sup>2+</sup>   | 4, 0.25  | 4, 0.18 | 4, 0.16 | 4, 0.13 | 4        | 4   | 1      | 6  | 6   |

Predicted  $k$  and corresponding  $s_k(A)$  [mAU] for precise methods and  $k$  for approximate methods. 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; R.S.D., residual standard deviation; RMS, root mean square error; AE, average error criterion;  $\chi^2$ , Bartlett  $\chi^2$  criterion; RPV, Scree test;  $\psi$ , Exner  $\psi$  function; IE, imbedded error function; IND, factor indicator function.

are calculated. Several instrumental methods have been devised for the second step, and depend on the chemical model of all equilibria and the 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 the first step towards further qualitative and quantitative analysis. Procedures for determining the chemical rank of a matrix comprising a variety of empirical and statistical methods based on principal component analysis (PCA), have been reported and critically compared [1–9]. 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 the various complexes.

The aim 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 three sulphoneazoxines with selected metal cations.

## 2. Theoretical

The application of various modifications of principal component analysis for determining the number of components that

Table 2  
The most probable chemical model in complex-forming equilibria of the *m*-CAPAZOXS + Me<sup>2+</sup> 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

|  | <i>m</i> -CAPAZOXS + Cd <sup>2+</sup> | <i>m</i> -CAPAZOXS + Co <sup>2+</sup> | <i>m</i> -CAPAZOXS + Ni <sup>2+</sup> | <i>m</i> -CAPAZOXS + Zn <sup>2+</sup> |
|--|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| $c_L$ [mol/L]  | 0.000221                              | 0.00022                               | 0.000187                              | 0.000218                              |
| Range $q_M$  | 0.05–0.88                             | 0.05–2.63                             | 0.05–5.00                             | 0.05–1.35                             |
| Range pH   | 4.759–4.777                           | 4.723–4.777                           | 4.690–4.778                           | 4.749–4.777                           |
| $n$  | 13                                    | 18                                    | 14                                    | 14                                    |
| $m$  | 28                                    | 28                                    | 28                                    | 28                                    |
| $n_c$  | 3                                     | 3                                     | 3                                     | 3                                     |
| $s_k(A)$ [mAU]   | 0.44                                  | 0.42                                  | 0.64                                  | 0.65                                  |
| Estimated stability constant, $\log \beta_{pqr}(s(\beta_{pqr}))$ |                                       |                                       |                                       |                                       |
| ML   | 6.59(21)                              | 7.19(6)                               | 7.64(7)                               | 4.83(3)                               |
| ML <sub>2</sub>  | 11.51(32)                             | 12.19(8)                              | 13.39(12)                             | 9.57(3)                               |
| The degree-of-fit test by the statistical analysis of residuals  |                                       |                                       |                                       |                                       |
| $\bar{e}$ [mAU]  | 0                                     | 0                                     | 0                                     | 0                                     |
| $ \bar{e} $ [mAU]  | 1.22                                  | 0.83                                  | 1.04                                  | 1.08                                  |
| $s(e)$ [mAU]   | 1.88                                  | 1.37                                  | 1.57                                  | 1.53                                  |
| $g_1(e)$ ( $\approx 0$ )   | –0.25                                 | 0.93                                  | –0.24                                 | –0.2                                  |
| $g_2(e)$ ( $\approx 3$ )   | 2.59                                  | 7.54                                  | 3.78                                  | 2.27                                  |
| $R$ [%] (<0.5)   | 0.44                                  | 0.34                                  | 0.38                                  | 0.37                                  |

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 the 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*:  $c_L$  (*m*-CAPAZOXS),  $I = 0.1$  (NaClO<sub>4</sub> + Na<sub>3</sub>PO<sub>4</sub> + HClO<sub>4</sub>), 25 °C, path length 1.000 cm,  $s_{\text{inst}}(A) = 0.45$  mAU (Specord M40, Zeiss, Jena Germany).

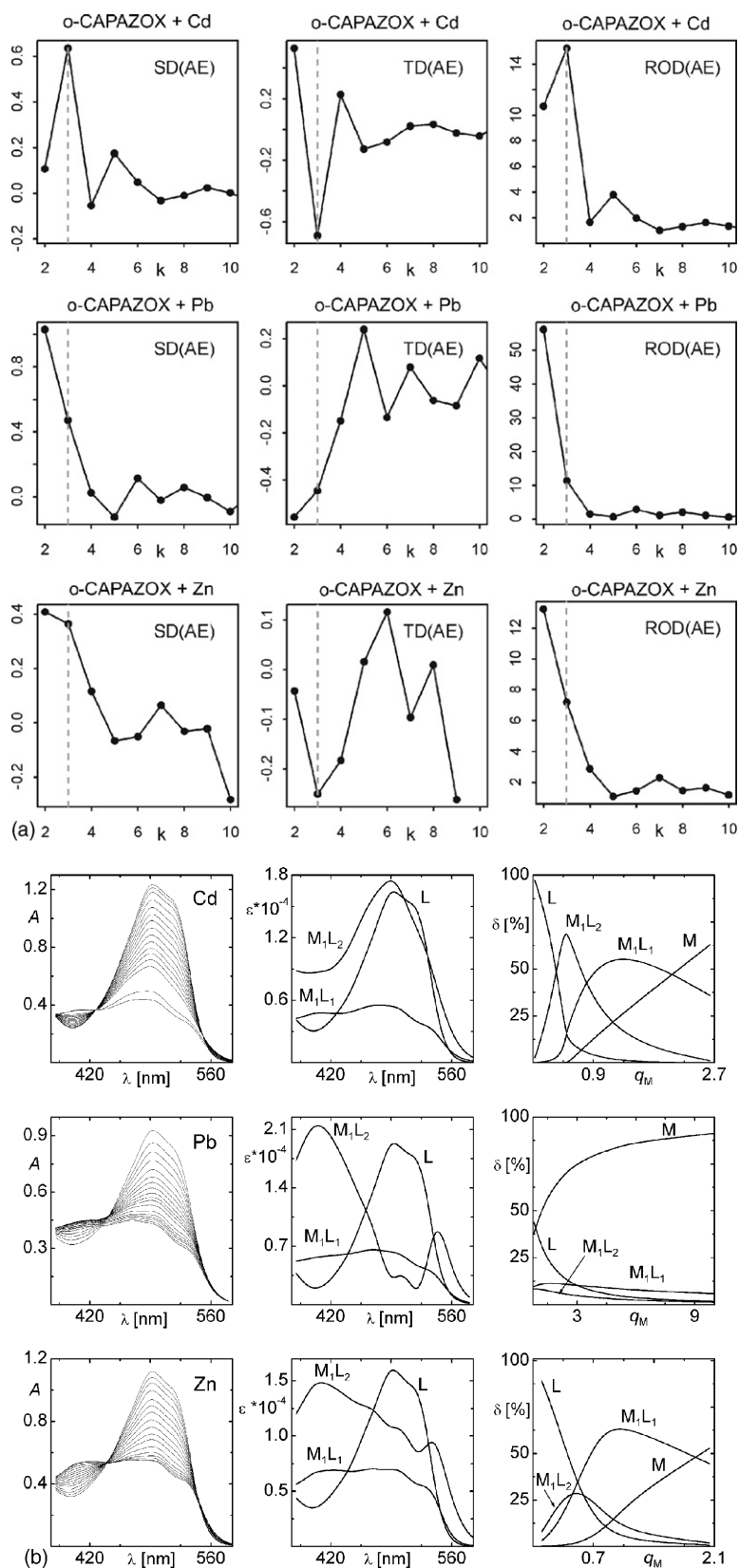


Fig. 1. (a) The derivatives detection criteria SD(AE), TD(AE) and ROD(AE) applied to the absorbance data set of complex-forming equilibria from the system of *o*-CAPAZOX and Cd<sup>2+</sup> in the first row, *o*-CAPAZOX and Pb<sup>2+</sup> in the second row and *o*-CAPAZOX and Zn<sup>2+</sup> in the third row. (b) Complex-forming equilibria of the *o*-CAPAZOX systems with three metal ions presented on the absorption spectra at 25 °C (left), the spectra of molar absorptivities vs. wavelengths for all of the complex species (middle) and the distribution diagram of the relative concentrations of all of the complex species in equilibrium: (first row) *o*-CAPAZOX + Cd<sup>2+</sup>, (second row) *o*-CAPAZOX + Pb<sup>2+</sup> and (third row) *o*-CAPAZOX + Zn<sup>2+</sup>.

contribute to the absorption spectra of multicomponent systems has been described in a previous paper for this journal [1]. All notation and computations relating to the determination of stability constants were performed by regression analysis of the spectra and have been described previously [1].

### 3. Experimental

The actual concentration of sulphoazoxine was determined by emf titration with NaOH and evaluated by regression program analysis with the ESAB [18] and MAGEC [19] programs. The purity of *o*-, *m*- and *p*-CAPAZOXS has previously been described in this way [8]. The impurities were mostly inorganic salts. Other solutions, the apparatus and the procedure of spectrophotometric titration were described in earlier papers [1,20].

## 4. Results and discussion

### 4.1. The number of complex species

Determination of the number of light-absorbing components in an equilibrium mixture is an important point prior to the formulation of the hypothesis of a chemical model. When the absorbance matrix monitoring the complex-forming equilibria of CAPAZOXS with various metal cations was subjected to PCA indices analysis using covariance about the origin, nine characteristics  $s_{\text{inst}}(A)$ , R.S.D., RMS, AE,  $\chi^2$ ,  $\psi$ , 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 applied to solve this problem may be divided into precise

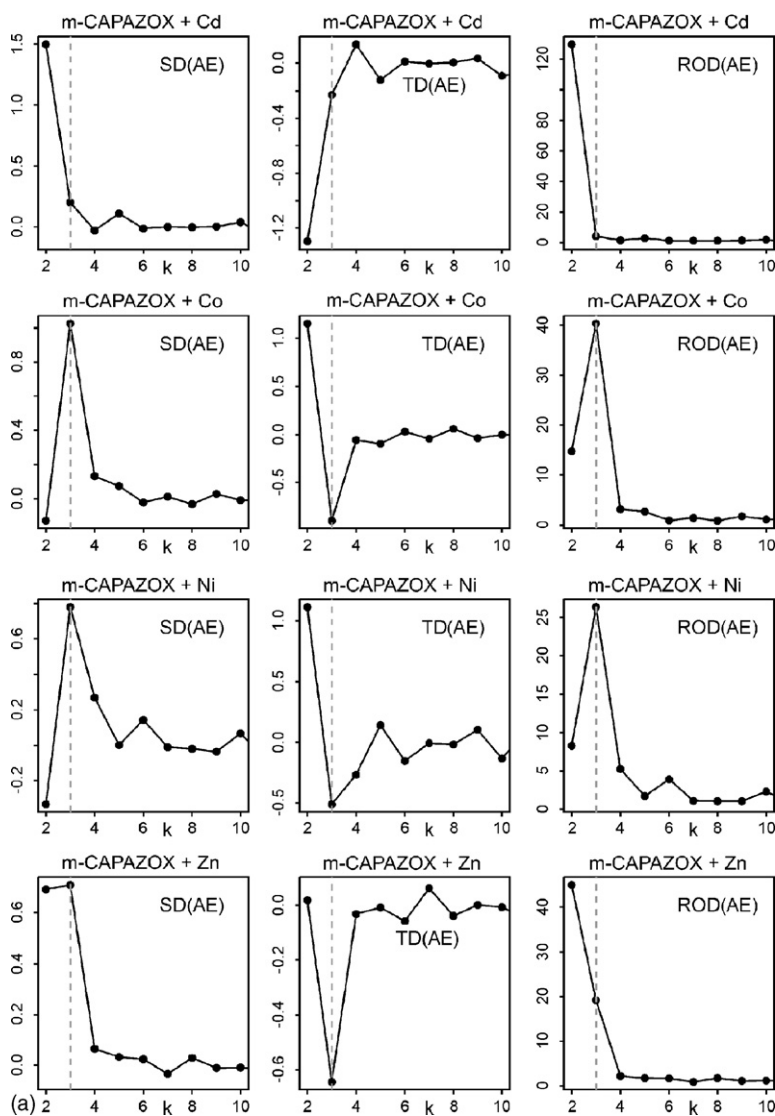


Fig. 2. (a) The derivatives detection criteria SD(AE), TD(AE) and ROD(AE) applied to the absorbance data set of complex-forming equilibria from the system of *m*-CAPAZOXS and  $\text{Cd}^{2+}$  in the first row, *m*-CAPAZOXS and  $\text{Co}^{2+}$  in the second row, *m*-CAPAZOXS and  $\text{Ni}^{2+}$  in the third row and *m*-CAPAZOXS and  $\text{Zn}^{2+}$  in the fourth row. (b) Complex-forming equilibria of the *m*-CAPAZOXS systems with four metal ions presented on the absorption spectra at 25 °C (left), the spectra of molar absorptivities vs. wavelengths for all of the complex species (middle) and the distribution diagram of all of the distribution concentrations of all of the complex species in equilibrium: (first row) *m*-CAPAZOXS +  $\text{Cd}^{2+}$ , (second row) *m*-CAPAZOXS +  $\text{Co}^{2+}$ , (third row) *m*-CAPAZOXS +  $\text{Ni}^{2+}$  and (fourth row) *m*-CAPAZOXS +  $\text{Zn}^{2+}$ .

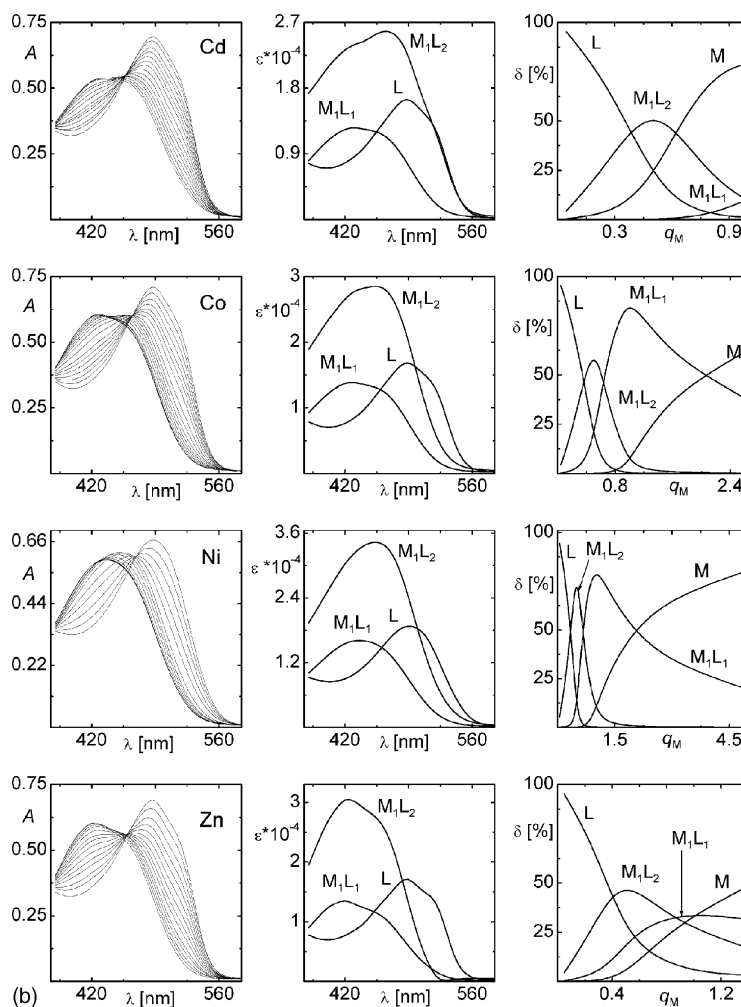


Fig. 2. (Continued).

methods –  $s_{\text{inst}}(A)$ , R.S.D., RMS and AE based on a knowledge of the experimental error of absorbance of spectrophotometer used  $s_{\text{inst}}(A)$  – and approximate methods ( $\chi^2$ ,  $\psi$ , RPV, IE and IND) requiring no knowledge of this experimental error [2–17]. Obviously, methods in the first category are preferred when the error is known [6–10]. The number of light-absorbing components  $n_c$  can be estimated from indices by comparing them with the experimental error, using the noise level  $s_{\text{inst}}(A)$ , R.S.D.<sub>inst</sub>, RMS<sub>inst</sub> and AE<sub>inst</sub> 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. The average error criterion AE seems to be one of the best and most reliable indices and the index value AE( $k$ ) was used for elucidation. Comparing an index value, AE( $k$ ) calculated for the variable index  $k$  to the estimated experimental error: if the AE( $k$ ) is greater than the estimated error AE<sub>inst</sub>, then the number of factors  $k$  is insufficient. If the AE( $k$ ) is approximately equal to the estimated error AE<sub>inst</sub>, then the proper number of factors  $k$  is appropriate. Generally, the dependence PC( $k$ ) =  $f(k)$  decreases steeply with an 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 (Table 1). The number of complex species in the mixture was evaluated more reliably with the use of the second derivatives SD(AE), the third derivatives TD(AE) and the derivatives ratio ROD(AE), using the following rules (Figs. 1–3): (a) the curve of the second derivative of the index function SD(AE( $k$ )) =  $f(k)$  exhibits a maximum for the true index  $k = n_c$ . (b) The curve of the third derivative of the index function TD(AE( $k$ )) =  $f(k)$  crosses 0 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(AE( $k$ )) =  $f(k)$  should reach a first maximum at the point where  $k = n_c$ . Tables 1–3 show the predicted number of complex species of the three CAPAZOXS complex-forming systems with selected metal cations, using the methods in Figs. 1–3.

#### 4.2. Complexation equilibria of *o*-, *m*- and *p*-CAPAZOXS

Multicomponent spectral analysis SQUAD(84) and  $\beta_{pqr}$  and  $\varepsilon_{pqr}$  adjustment for a given spectra by fitting the predicted absorbance-response surface to given spectral data, with one dimension representing the dependent variable (absorbance), and the other two dimensions representing the independent

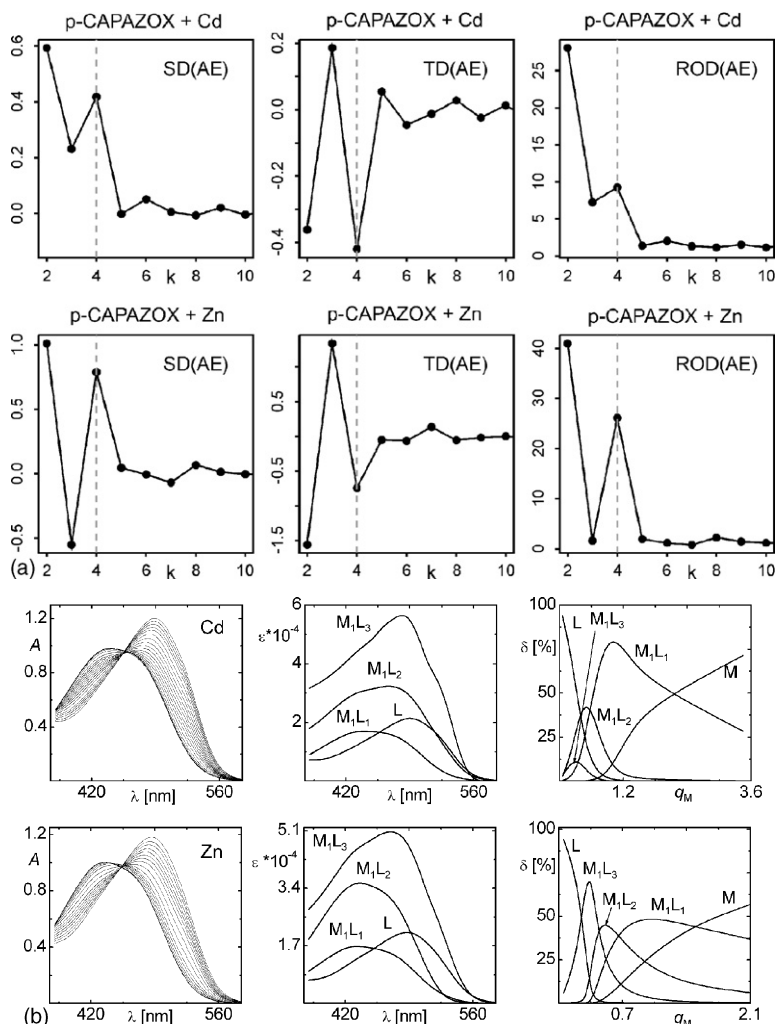


Fig. 3. (a) The derivatives detection criteria SD(AE), TD(AE) and ROD(AE) applied to the absorbance data set of complex-forming equilibria from the system of *p*-CAPAZOXs and Cd<sup>2+</sup> in the first row and *p*-CAPAZOXs and Zn<sup>2+</sup> in the second row. (b) Complex-forming equilibria of the *p*-CAPAZOXs systems with two metal ions presented on the absorption spectra at 25 °C (left), the spectra of molar absorptivities vs. wavelengths for all of the complex species (middle) and the distribution diagram of the relative concentrations of all of the complex species in equilibrium: (first row) *p*-CAPAZOXs + Cd<sup>2+</sup> and (second row) *p*-CAPAZOXs + Zn<sup>2+</sup>.

variables (the total component concentrations  $n_s$  solutions at  $n_w$  wavelengths). The parameters to be determined are [13,17]: (i) the number of species  $n_c$  and their stoichiometric indices  $(p, q, r)_j, j = 1, \dots, n_c$ , (ii) their stability constants  $\beta_{pqr}$  and molar absorptivities  $\varepsilon_{pqr}$  and (iii) the free concentrations  $[M_pL_qH_r]_j, j = 1, \dots, n_c$ , of all the species in the chemical model proposed. The general equations for complexes are  $pM + qL + rH = M_pL_qH_r$  with  $\beta_{pqr} = [M_pL_qH_r]/([M]^p [L]^q [H]^r)$ , where charges are omitted for simplicity. For the  $i$ th solution and  $k$ th wavelength, the absorbance  $A_{ik}$  is given by relation  $A_{ik} = \sum_{j=1}^{n_c} (\varepsilon_{pqr,k} \beta_{pqr} [M]^p [L]^q [H]^r)_j$  where  $\varepsilon_{pqr,k}$  is the molar absorptivity of species  $[M_pL_qH_r]$  at the  $k$ th wavelength. When the estimated  $\beta_{pqr}$  and  $\varepsilon_{pqr}$  values have been refined, the agreement between the experimental and predicted data can be examined. The residuals are analysed to discover whether the refined parameters adequately represent the data. To analyse the residuals a degree-of-fit test is performed by the statistical analysis of residuals (Tables 2 and 3): the residual mean  $\bar{e}$  should be nearly 0, the mean residual  $|\bar{e}|$  and the standard deviation of

residuals  $s(e)$  should be at the same level as the instrumental noise  $s_{inst}(A)$ , the residual skewness  $g_1(e)$  should be 0 so proving a symmetric distribution and  $g_2(e)$  should be 3, so proving the Gaussian distribution of residuals, and finally the Hamilton  $R$ -factor for relative fit  $R$  [%] should be less than 0.5% for an excellent fitness achieved (Tables 2 and 3).

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 CAPAZOXs mostly 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  $ML_3$  can also exist, while in equimolar solutions only  $ML_2$  and  $ML$  complexes are present. Table 2 presents the results of the chemical model determination for the *m*-CAPAZOXs system and Table 3 for the *o*- and *p*-CAPAZOXs system. The chemical model concerns a guess as to the number of light-absorbing species  $n_c$  coexisting in the equilibrium mixture, their stoichiometry, their stability constants

Table 3

The most probable chemical model in complex-forming equilibria of the *o*- and *p*-CAPAZOXS + Me<sup>z+</sup> 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

|   | <i>o</i> -CAPAZOXS + Cd <sup>2+</sup> | <i>o</i> -CAPAZOXS + Pb <sup>2+</sup> | <i>o</i> -CAPAZOXS + Zn <sup>2+</sup> | <i>p</i> -CAPAZOXS + Cd <sup>2+</sup> | <i>p</i> -CAPAZOXS + Ni <sup>2+</sup> | <i>p</i> -CAPAZOXS + Zn <sup>2+</sup> |
|---|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| $c_L$ [mol/L]   | 0.000391                              | 0.0000234                             | 0.000397                              | 0.000293                              | 0.000278                              | 0.000297                              |
| Range $q_M$   | 0.03–2.63                             | 0.8–33.33                             | 0.10–2.04                             | 0.06–3.45                             | 0.04–6.25                             | 0.05–2.5                              |
| Range pH  | 4.685–4.777                           | 2.981–2.966                           | 4.703–4.774                           | 4.685–4.777                           | 4.627–4.778                           | 4.709–4.777                           |
| $n$   | 16                                    | 18                                    | 17                                    | 20                                    | 15                                    | 18                                    |
| $m$   | 28                                    | 21                                    | 28                                    | 28                                    | 28                                    | 28                                    |
| $n_c$   | 3                                     | 3                                     | 3                                     | 4                                     | 4                                     | 4                                     |
| $s_k(A)$ [mAU]  | 0.54                                  | 0.3                                   | 1.03                                  | 0.24                                  | 0.76                                  | 0.18                                  |
| Estimated stability constant, log $\beta_{pqr}(s(\beta_{pqr}))$ |                                       |                                       |                                       |                                       |                                       |                                       |
| ML  | 6.39(5)                               | 4.24(2)                               | 5.18(7)                               | 6.44(5)                               | 7.72(18)                              | 6.84(16)                              |
| ML <sub>2</sub>   | 11.51(9)                              | 9.01(2)                               | 9.06(10)                              | 10.99(10)                             | 13.86(34)                             | 13.05(29)                             |
| ML <sub>3</sub>   | –                                     | –                                     | –                                     | 14.57(25)                             | 17.88(63)                             | 18.74(43)                             |
| The degree-of-fit test by the statistical analysis of residuals |                                       |                                       |                                       |                                       |                                       |                                       |
| $\bar{e}$ [mAU]   | 0                                     | 0.000002                              | 0                                     | 0                                     | 0                                     | 0                                     |
| $ \bar{e} $ [mAU]   | 1.03                                  | 0.99                                  | 1.67                                  | 0.69                                  | 1.03                                  | 1                                     |
| $s(e)$ [mAU]  | 1.83                                  | 1.49                                  | 2.68                                  | 1.2                                   | 1.73                                  | 1.76                                  |
| $g_1(e)$ ( $\approx 0$ )  | 0.59                                  | –0.39                                 | 0.41                                  | 1.04                                  | 0.4                                   | 0.23                                  |
| $g_2(e)$ ( $\approx 3$ )  | 6.02                                  | 3.67                                  | 4.11                                  | 6.84                                  | 3.83                                  | 3.8                                   |
| $R$ [%] (<0.5)  | 0.34                                  | 0.3                                   | 0.5                                   | 0.17                                  | 0.24                                  | 0.25                                  |

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*:  $c_L$  (*o*-, *p*-CAPAZOXS),  $I = 0.1$  (NaClO<sub>4</sub> + Na<sub>3</sub>PO<sub>4</sub> + HClO<sub>4</sub>), 25 °C, path length 1.000 cm,  $s_{inst}(A) = 0.45$  mAU (Specord M40, Zeiss, Jena Germany).

estimated by regression analysis and at the same time the curves of molar absorption coefficients dependent on wavelength. For a set of current values of  $\beta_{pqr}$  the free concentrations of metal [M] and ligand [L], followed by the concentrations of all the complexes in the equilibrium mixture  $[M_pL_qH_r]_j$ ,  $j = 1, \dots, n_c$ , for  $n$  solutions the matrix **C**, and are presented in the form of a distribution diagram of relative concentrations in Figs. 1b, 2b and 3b.

In addition to the fit achieved, it is also necessary to examine the physico-chemical sense of the model parameter estimates, such as positive values in the concentration matrix, positive values of molar absorptivities and 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 merely as “noise”, 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<sub>2</sub> 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 not obtained for *o*- and *m*-CAPAZOXS. Therefore, the final chemical model contained three light-absorbing components ML, ML<sub>2</sub> and L in most systems for *o*- and *m*-derivates. However, *p*-CAPAZOXS exhibits four species in equilibrium ML, ML<sub>2</sub>, ML<sub>3</sub> and L.

Cherkesov [21] has explained the formation of a covalent bond of the central metal ion to the azo group nitrogen. 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. 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.

## 5. Conclusion

1. Generally, the most reliable index methods seem to be those based on a knowledge of the experimental error of absorbance. The average error criterion seems to be the most reliable method for the determination of the instrumental standard deviation of the spectrophotometer used.
2. Index methods are all based on finding the point at which the slope of the index function changes, and therefore the application of derivatives is very useful. 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  $s_{inst}(A)$  are preferred as the more reliable procedure, as they always predict the correct number of components and even the presence of minor species in the 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  $25^\circ\text{C}$ . The standard deviations of the estimated stability constant,  $\log \beta_{pqr}(s(\beta_{pqr}))$  of the last valid digits in brackets are in brackets:

*o*-CAPAZOXS +  $\text{Cd}^{2+}$ : For  $q_M$  0–2.6 leading to three light-absorbing species L, ML and  $\text{ML}_2$  with estimates of stability constants  $\log \beta_{110} = 6.39(5)$  and  $\log \beta_{120} = 11.51(9)$ .

*o*-CAPAZOXS +  $\text{Pb}^{2+}$ : For  $q_M$  1–33 leading to three light-absorbing species L, ML and  $\text{ML}_2$  with  $\log \beta_{110} = 4.24(2)$  and  $\log \beta_{120} = 9.01(2)$ .

*o*-CAPAZOXS +  $\text{Zn}^{2+}$ : For  $q_M$  0–53 leading to three light-absorbing species L, ML and  $\text{ML}_2$  with  $\log \beta_{110} = 5.18(7)$  and  $\log \beta_{120} = 9.06(10)$ .

*m*-CAPAZOXS +  $\text{Cd}^{2+}$ : For  $q_M$  0–1 leading to three light-absorbing species L, ML and  $\text{ML}_2$  with  $\log \beta_{110} = 6.59(20)$  and  $\log \beta_{120} = 11.51(32)$ .

*m*-CAPAZOXS +  $\text{Co}^{2+}$ : For  $q_M$  0–2.6 leading it lead to three light-absorbing species L, ML and  $\text{ML}_2$  with  $\log \beta_{110} = 7.19(6)$  and  $\log \beta_{120} = 12.19(8)$ .

*m*-CAPAZOXS +  $\text{Ni}^{2+}$ : For  $q_M$  0–5 it leading three light-absorbing species L, ML and  $\text{ML}_2$  with  $\log \beta_{110} = 7.64(7)$  and  $\log \beta_{120} = 13.39(12)$ .

*m*-CAPAZOXS +  $\text{Zn}^{2+}$ : For  $q_M$  0–1.4 leading to three light-absorbing species L, ML and  $\text{ML}_2$  with  $\log \beta_{110} = 4.83(3)$  and  $\log \beta_{120} = 9.57(3)$ .

*p*-CAPAZOXS +  $\text{Cd}^{2+}$ : For  $q_M$  0–3.5 leading to three light-absorbing species L, ML,  $\text{ML}_2$  and  $\text{ML}_3$  with  $\log \beta_{110} = 6.44(5)$ ,  $\log \beta_{120} = 10.99(10)$  and  $\log \beta_{130} = 14.57(25)$ .

*p*-CAPAZOXS +  $\text{Zn}^{2+}$ : For  $q_M$  0–2.5 leading to four light-absorbing species L, ML,  $\text{ML}_2$  and  $\text{ML}_3$  with  $\log \beta_{110} = 6.84(16)$ ,  $\log \beta_{120} = 13.05(29)$  and  $\log \beta_{130} = 18.74(43)$ .

## Acknowledgements

The financial support of the Internal Grant Agency of the Czech Ministry of Health (grant no. NR 9055-4/2006) and of the Czech Ministry of Education (grant no. MSMT 0021627502) is gratefully acknowledged.

## References

- [1] M. Meloun, T. Čapek, T. Syrový, Talanta 66 (2005) 547.
- [2] E.R. Malinowski, Factor Analysis in Chemistry, second ed., Wiley, New York, 1991.
- [3] M. Meloun, J. Havel, E. Högfeltd, Computation of Solution Equilibria, Horwood, Chichester, 1988.
- [4] E.R. Malinowski, J. Chemom. 13 (1999) 69.
- [5] E.R. Malinowski, Anal. Chem. 49 (1977) 612.
- [6] A.K. Elbergali, J. Nygren, M. Kubista, Anal. Chim. Acta 379 (1999) 143.
- [7] J.M. Dean, in: R.G. Brereton (Ed.), Multivariate Pattern Recognition in Chemometrics Illustrated by Case Studies, Elsevier, Amsterdam, 1992.
- [8] M. Meloun, J. Čapek, P. Mikšík, R.G. Brereton, Anal. Chim. Acta 423 (2000) 51.
- [9] M. Meloun, K. Kupka, J. Čapek, T. Syrový, INDICES Algorithm, <http://meloun.upce.cz> and the block Algorithms.
- [10] J.J. Kankare, Anal. Chem. 42 (1970) 1322.
- [11] M.S. Bartlett, Br. J. Psych. Stat. Sec. 2 (1950) 77.
- [12] R.D. Catell, Multivar. Behav. Res. 1 (1966) 245.
- [13] (a) D.J. Leggett (Ed.), Computational Methods for the Determination of Formation Constants, Plenum Press, New York, 1985, pp. 99–157; (b) D.J. Leggett (Ed.), Computational Methods for the Determination of Formation Constants, Plenum Press, New York, 1985, pp. 291–353; (c) J. Havel, M. Meloun, Computational Methods for the Determination of Formation Constants, Plenum Press, New York, 1985, p. 19; (d) J. Havel, M. Meloun, Computational Methods for the Determination of Formation Constants, Plenum Press, New York, 1985, p. 221.
- [14] D.J. Leggett, W.A.E. McBryde, Anal. Chem. 47 (1975) 1065.
- [15] M. Meloun, M. Javůrek, J. Havel, Talanta 33 (1986) 513, available online at <http://meloun.upce.cz>.
- [16] M. Meloun, J. Havel, E. Högfeltd, Computation of Solution Equilibria, Ellis Horwood, Chichester, 1988.
- [17] (a) M. Meloun, J. Militký, M. Forina, PC-Aided Regression and Related Methods, vol. 2, Ellis Horwood, Chichester, 1994; (b) M. Meloun, J. Militký, M. Forina, PC-Aided Statistical Data Analysis, vol. 1, Ellis Horwood, Chichester, 1992.
- [18] (a) C. Rigano, M. Grasso, S. Sammartano, Ann. Chim. Rome 74 (1984) 537; (b) C. De Stefano, P. Princi, C. Rigano, S. Sammartano, Ann. Chim. Rome 77 (1987) 643.
- [19] P.M. May, D.R. Williams, P.W. Linder, R.G. Torrington, Talanta 29 (1982) 249.
- [20] M. Meloun, V. Říha, J. Žáček, Chem. Listy 82 (1988) 765, available online at <http://meloun.upce.cz>.
- [21] A.I. Cherkosov, Zh. Anal. Khim. 17 (1962) 16.