J. Chem. Eng. Data **2007,** *52,* 1171−1178

Study of Complex Formation between 4-(2-Pyridylazo) Resorcinol and Al^{3+}, Fe^{3+}, Zn^{2+}, and Cd^{2+} Ions in an Aqueous Solution at 0.1 M Ionic Strength

J. Ghasemi,*,[†] H. Peyman,[†] and M. Meloun[‡]

Chemistry Department, Faculty of Sciences, Razi University, Kermanshah, Iran, and Department of Analytical Chemistry, University of Pardubice, Pardubice, Czech Republic

The complexation reactions between 4-(2-pyridylazo)resorcinol (PAR) and Al^{3+}, Fe^{3+}, Zn^{2+}, and Cd^{2+} ions at 25 °C and 0.1 M ionic strength are performed spectrophotometrically. A critical comparison of the various PCA methods on the absorbance matrix data concerning the number of light-absorbing species has been made. Knowing the number of light-absorbing species is a critical step for subsequent quantitative and qualitative solution equilibria studies. Therefore, the nine selected index functions for the prediction of the number of light-absorbing components which contribute to a set of spectra are critically tested using the algorithms implemented in INDICES software. Behind the number of various complexes formed the stability constants of species ML and MLH, type log β_{110} and log β_{111}, for the system of PAR (ligand L) with Zn^{2+} and Cd^{2+} ions and the stability constants of species MLH and ML_2H, type log β_{111} and log β_{121}, for the system of PAR (ligand L) with Al^{3+} and Fe^{3+} ions at 25 °C and 0.1 M ionic strength, and various mole ratios are estimated by the SQUAD and EQUISPEC programs.

1. Introduction

4-(2-Pyridylazo)resorcinol (PAR) has the reactive groups (a heterocyclic nitrogen group, azo group, and *o*-hydroxyl group as shown in Figure 1) available for possible coordination to metal ions. PAR is a dibasic acid and simultaneously forms the protonated and the normal type of complexes with most metal ions.[1] The complex-forming equilibria and the stability constant of PAR with various metal cations in various mole ratios 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 the stability constants of all the complex species involved are calculated. Several instrumental methods have been devised for the second step and depend on the chemical method of all equilibria and experimental designs. 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.[2−8] 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 PAR with four metal cations. In the second step, we obtained the stability constant. The most widespread programs and algorithms for determining the stability constant from absorbance data are SQUAD and EQUISPEC.

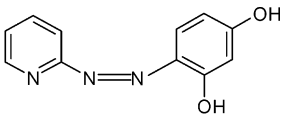

Figure 1. Structure of PAR.

2. Theoretical

Principal component analysis has been used in a powerful fashion for determining the number of components that contributes to the absorption spectra of multicomponent systems.[2,8]

2.1. Notation. The absorbance $A_{i,j}$ is the element of the absorbance matrix **A** of size $n\cdot m$ being measured for n solutions with known total concentrations of three basic components, c_M, c_L, and c_H, at m wavelengths. Throughout this work, it is assumed that the $n\cdot m$ absorbance data matrix $\mathbf{A} = \epsilon\mathbf{C}$ containing the n recorded and digitized spectra as rows can be written as the product of the $m\cdot n_c$ matrix of molar absorptivities ϵ and the $n_c\cdot n$ concentration matrix **C**. Here, n_c is the number of components that absorb in the chosen spectral range. The rank of the matrix **A** is obtained from the equation rank $(\mathbf{A}) = \min[\text{rank}(\epsilon), \text{rank}(\mathbf{C})] \leq \min(m, n_c, n)$. Because the rank of **A** is equal to the rank of ϵ or **C**, whichever is smaller, and because rank $(\epsilon) \leq n_c$ and rank $(\mathbf{C}) \leq n_c$, and provided that m and n are equal to or greater than n_c, it will only be necessary to determine the rank of matrix **A** which is equivalent to the number of dominant light-absorbing components.[6−8]

2.2. Determination of the Number of Complex Species. Meloun et al.[8] have introduced an excellent program, INDICES, for the determination of the number of light-absorbing species in the recorded spectral data matrix. They presented various

---

* Corresponding author. E-mail: Jahan.ghasemi@gmail.com.
[†] Razi University.
[‡] University of Pardubice.

10.1021/je060325g CCC: \$37.00

indicator functions to deduce the exact size of the true component space which 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.[7,8] Elbergali et al.[9] have also reviewed several indicator functions which are commonly used to assign the significant factors in the PC decomposition process. In the INDICES program which can be run in the S-PLUS programming environment, 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 the spectrophotometer used, the corresponding k represents the number of light-absorbing components in a mixture, $n_c = k$.

2.2.1. Precise Indices. Precise index methods[10] are based on a comparison of an actual index of the method used with the experimental error of the instrument used. The Kankare residual standard deviation method,[8,11] the residual standard deviation, rsd(k), method,[2,8] the root-mean-square error, rms$(k)^2$, method which is closely related to rsd(k), the average error criterion AE(k) method,[2,8] and the Bartlett χ^2 criterion, $\chi^2(k)$, method[2,8] belong to precise methods.

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. 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, that $\psi = 0.2$ can be considered a good correlation, and that $\psi = 0.1$ can be considered 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. The Scree, RPV(k), test for identifying the true dimensionality of a data set, proposed by Cattell,[12] 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.[2,12] The imbedded error function, IE(k), and the factor indicator function, IND(k), are presented by Malinowski[2] and are empirical functions and reach a minimum when the correct number of latent variables or PC dimensions has been employed in the data reproduction.

2.3. Determination of the Chemical Model in Complex-Forming Equilibria. If the complex-forming equilibria between a metal, M (the charges are omitted for the sake of simplicity), a ligand, L, and a proton, H, are 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, $(p, q, r)_i$, $i = 1, ..., n_c$, their stoichiometry, then the overall stability constant, β_{pqr}, may be expressed as

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

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

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

where $\epsilon_{pqr,j}$ is the molar absorptivity of the $M_pL_qH_r$ species with stoichiometric coefficients p, q, and r measured at the jth wavelength. The multicomponent spectra analyzing programs SQUAD(84)[13−15] and EQUISPEC[16] can adjust β_{pqr} and ϵ_{pqr} for absorption spectra.

The SQUAD (stability quotients from absorbance data) program was written in FORTRAN by Leggett,[14,17] and for the first time a factor analysis method in the program for determination of stability constants was used. The calculation of the best values of the stability constants was carried out by employing a nonlinear least-squares approach. The nonlinear least-squares method used is based on the minimization of the function, U

$$U = \sum_{i=1}^{n}\sum_{j=1}^{m}(A_{\text{exptl},i,j} - A_{\text{calcd},i,j})^2 = \sum_{i=1}^{n}\sum_{j=1}^{m}(A_{\text{exptl},i,j} - \sum_{k=1}^{n_c}\epsilon_{j,k}[M_pL_qH_r]_k)^2 = \min \qquad (3)$$

where $A_{i,j}$ represents the element of the experimental absorbance response plane of size $n{\cdot}m$ 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 experimental and computational scheme for the determination of the stability constants of a multicomponent system was taken from Meloun et al.[18] Various hypotheses of chemical models with refined parameters have been tested, and the statistical characteristics describing the degree-of-fit of regression spectra through experimental points have been calculated. The calculated standard deviation of absorbance $s(A)$ was used as the most important criterion for a fitness test. If after termination of the minimization process the condition $s(A) \approx s_{\text{inst}}(A)$ is met, the hypothesis of the chemical model is taken as the most probable and is accepted.[3,18]

In 1997, Maeder et al. introduced the EQUISPEC program using the matrix-based MATLAB environment for global analysis of spectrophotometric equilibrium data.[16,19−22] In a spectrophotometric titration, it is most convenient to arrange the series of measured spectra as the rows of a matrix **A**.

A generally applicable method for the investigation of systems which suffer from rank deficient data sets and/or poorly defined equilibria of minor species has been published recently.[23] The method involves simultaneous global analysis of a series of spectrophotometric titrations, each performed under different initial conditions or concentrations, that they called second-order global analysis. The term globalization has been coined for the analysis of multichannel/multiwavelength data sets.[24−27]

3. Experimental

3.1. Chemicals and Solutions. All the chemicals were of analytical reagent grade. Five stock 4-(2-pyridylazo)resorcinol solutions of metal ions, from their nitrate salts, were prepared in a 100 mL volumetric flask by direct weighing of the required amounts of commercially available reagents (all from Merck), then by dissolving in doubly distilled water, and the solutions were used for preparation of the various mole ratio mixtures of PAR. Titration of each solution was carried out at a fixed ionic strength of 0.1 M with $NaClO_4$ solution. The starting points of titrations were pH = 2, which were set using concentrated solutions of HCl. The concentrated NaOH solution was also used for titrations, to avoid dilution of the working solution. All experiments were carried out at 25 °C, and all titrations are repeated at least three times.

3.2. Apparatus and Software. The pH values were measured by a model 300 HANA pH meter using a combined glass electrode. The glass electrode was calibrated on the basis of

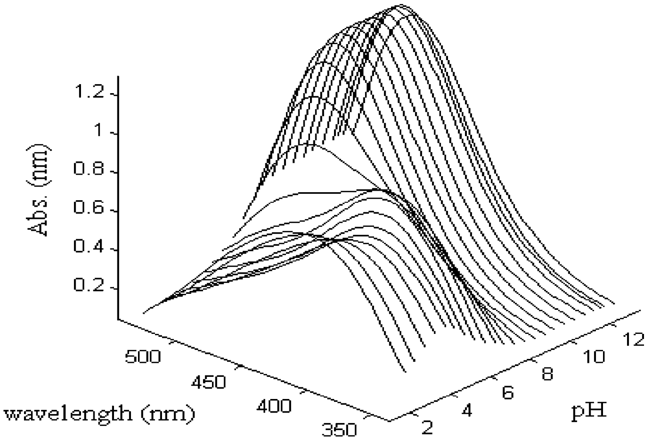

Figure 2. Absorbance spectra of the mixture of PAR with Cd^{2+} in a mole ratio of $C_M/C_L = 1$ and of different pH values.

the proton concentration at constant ionic strength according to the procedure described elsewhere.[28] The calibration procedure was as recommended by the IUPAC for glass electrodes.[29] Absorption spectra were measured on an Agilent 8453 UV−visible diode array spectrophotometer using the Agilent UV−visible ChemStation Software for data acquisition. A cell of 10 mm optical path was used for all measurements. The data were preprocessed using MATLAB software, version 6.5 (Mathworks, Natick, USA), and the deconvolution of the obtained data matrix was performed using the software INDICES, SQUAD, and EQUISPEC.

3.3. Supporting Information Available. A qualitative interpretation of the spectra with the use of the INDICES program aims to evaluate the quality of the data set, to remove spurious data, and to estimate the minimum number of factors, i.e., contributing aqueous species, which are necessary to describe the experimental data and determine the number of dominant species present in the equilibrium mixture. Complete computational procedures of the INDICES and SQUAD programs, input data specimen, and corresponding output in numerical and graphical form are available free of charge via the Internet at http://meloun.upce.cz in the block DATA and ALGORITHMS.

4. Results and Discussion

4.1. Absorption Spectra. The absorption spectra at 340−540 nm at three mole ratios of $C_M/C_L = 0.5$, 1, and 2 of metal cations and PAR at the pH range 2−12 were recorded. A sample absorption spectrum of the pH titration of PAR with Cd^{2+} is shown in Figure 2. The number of absorbing species and the stability constants of the resulting complexes are calculated from absorption data matrices at each metal to ligand ratio. The numbers of light-absorbing species are determined by several algorithms aforementioned in the Introduction section implemented in INDICES, which can be run in S-Plus software. In the second step, the overall stability constants of all resulting complexes between PAR and each metal ion in the studied pH

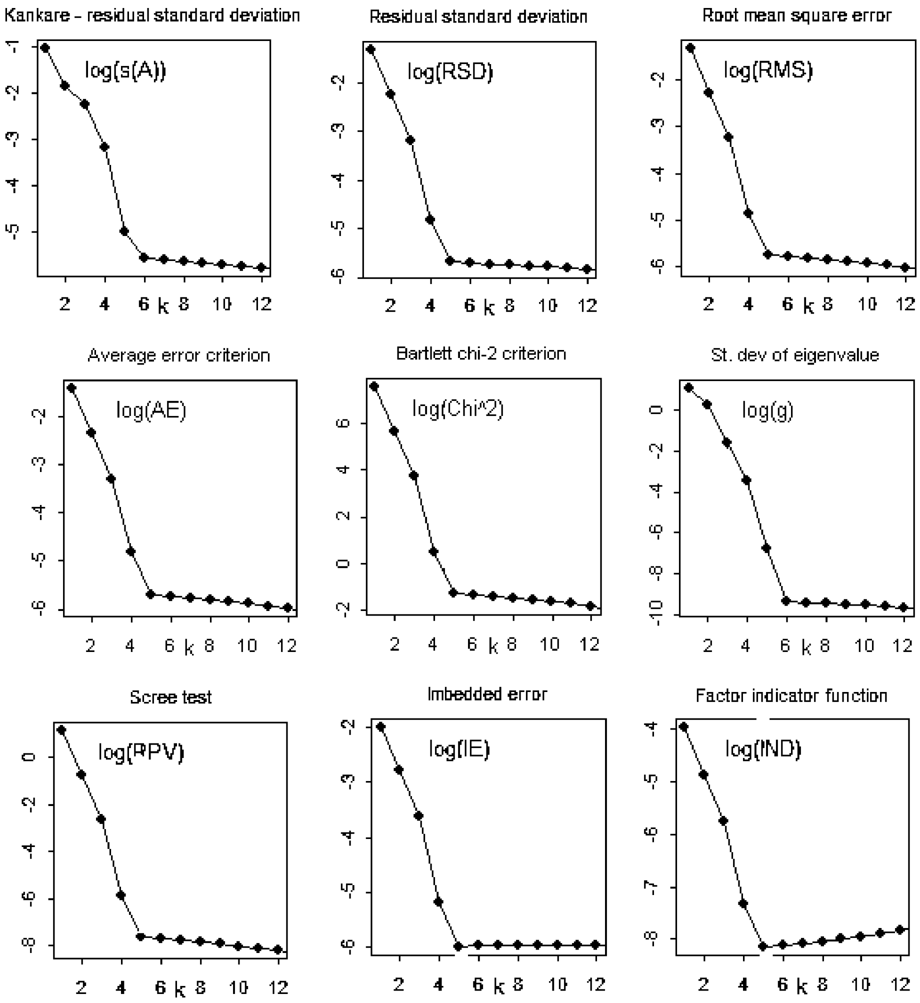

Figure 3. Logarithmic dependence of nine index methods as a function of the number of principal components *k* for the absorbance matrix of complex-forming equilibria of PAR with Zn^{2+} in $C_M/C_L = 2$.

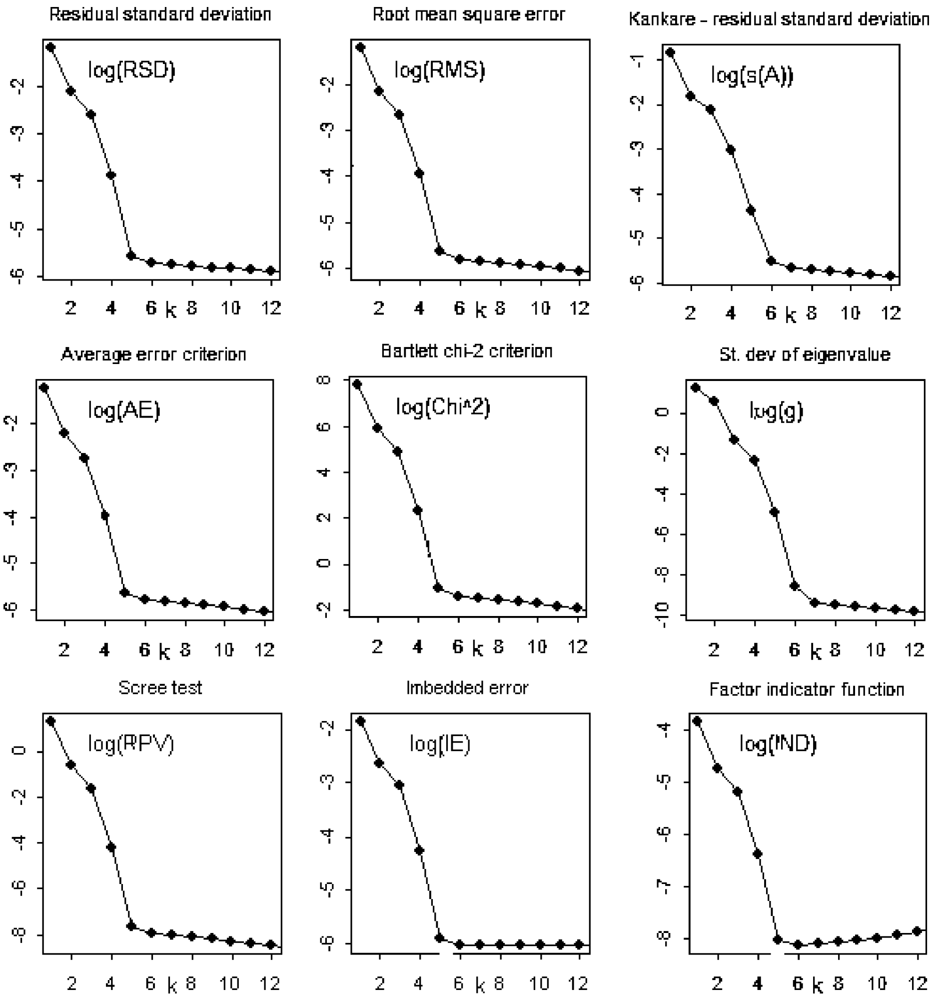

Figure 4. Logarithmic dependence of nine index methods as a function of the number of principal components *k* for the absorbance matrix of complex-forming equilibria of PAR with Cd^{2+} in $C_M/C_L = 2$.

range are calculated by the SQUAD and EQUISPEC programs. The outputs of the INDICES are variations of each specific function against the number of principal components, *k*. The outputs of the two data processing programs, SQUAD and EQUISPEC, in addition to the overall stability constants, contain standard deviations of the estimated constants, distribution diagrams, and spectral profiles of all species.

4.2. Number of Light-Absorbing 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 measurements are documented.[30] Colored impurities in PAR may have an acid−base character in which case the background color will vary with pH. Some indicators may separate from solution and/or be adsorbed on the cuvette walls. 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. The absorbance matrix monitoring the complex-forming equilibria of PAR with various metal cations was subjected to PCA index analysis using nine characteristics, $s_{inst}(A)$, rsd, rms, AE, $\chi 2$, ψ, RPV, IE, and IND. 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 the spectrophotometer used, $s_{inst}(A)$, and approximate methods (χ^2, ψ, RPV, IE, and IND) requiring no knowledge of this experimental error, $s_{inst}(A)$. 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 indices *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 (Figures 3 and 4) for the absorbance matrix of the PAR$-Zn^{2+}$ and $-Cd^{2+}$ complex-forming systems, and the $s_k(A)$, rsd, rms, and AE indices lead to $n_c = 5$. 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. For $k = 5$, the break appears, proving that five species are in the equilibrium mixture. For the scree test, $RPV(k) = f(k)$, the residual percentage variance RPV(*k*) plotted against the number

Table 1. Estimated Number of Light-Absorbing Species, $n_c = k$, $s_{inst}(A) = s_k(A)$, by the Logarithmic Technique of PCA Methods (INDICES Program) for the PAR Complex-Forming System with M^{n+} in Different Mole Ratios[a]

	predicted k for precise and approximate methods								
C_M/C_L	$s_k(A)$	rsd	rms	AE	χ^2	RPV	ψ	IE	IND
				Zn^{2+}					
0.5	7	6	6	6	6	6	7	6	6
1	6	5	5	5	5	5	6	5	5
2	6	5	5	5	5	5	6	5	5
				Cd^{2+}					
1	6	5	5	5	5	5	6	5	5
2	6	5	5	5	5	5	6	5	5
				Al^{3+}					
0.5	5	5	5	5	5	5	6	5	5
1	6	5	5	4	5	5	6	4	5
2	6	5	5	5	5	5	6	5	5
				Fe^{3+}					
0.5	6	5	5	5	6	6	6	5	5
1	5	4	5	5	5	5	5	4	5
2	5	5	5	6	5	5	5	5	5

[a] The $n \cdot m$ absorbance matrix is for $n = 22$ solutions with total concentrations of C_M, C_L, and C_H, with different mole ratios (C_M/C_L = 0.5, 1, and 2) and measured at $m = 41$ 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.

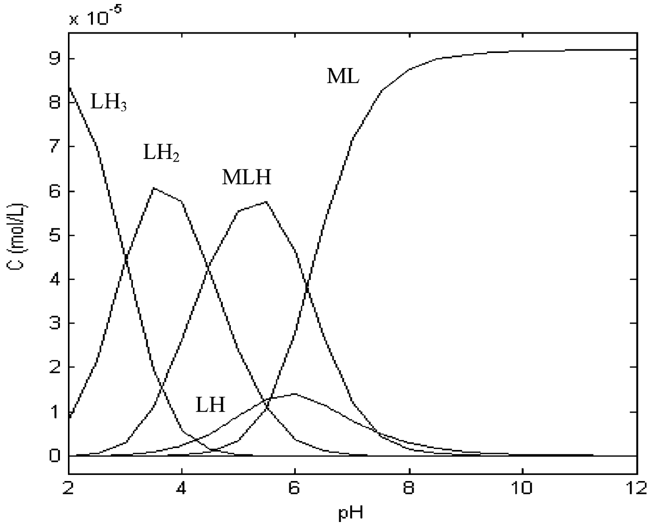

Figure 5. Distribution diagram of the relative concentration of all complex species in equilibrium between PAR and Zn^{2+} ($C_M/C_L = 1$).

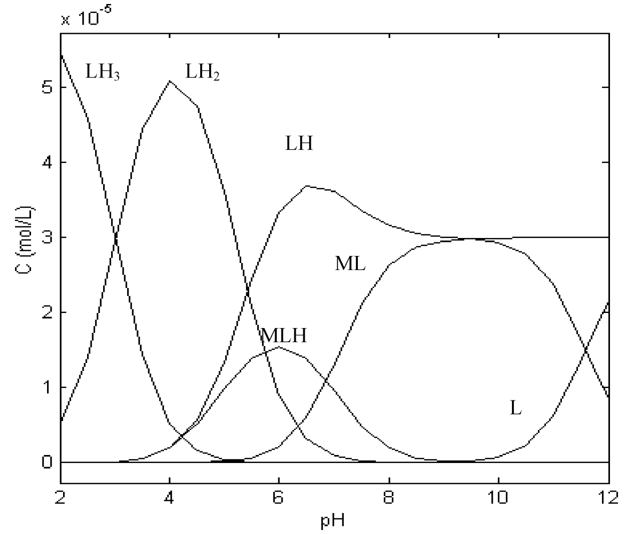

Figure 6. Distribution diagram of the relative concentration of all complex species in equilibrium between PAR and Cd^{2+} ($C_M/C_L = 0.5$).

of components k shows that the curve drops rapidly and levels off at $n_c = 5$. This point can be used to reduce the component space. From Figures 3 and 4, we observe that the minimum appears in the $s(k) = f(k)$ and $\psi(k) = f(k)$ functions at $k = 5$. The $s(k)$ in Figure 3 shows a progressive decrease on going from $k = 1$ to $k = 5$ with a break at $k = 5$. This hints that five species are responsible for the absorbance matrix. The $\psi(k) = f(k)$ function, similar to the $s(k) = f(k)$ function in Figure 4, reaches a minimum at $k = 5$ which is more pronounced and occurs in situations where $s(k) = f(k)$ exhibits no clear minimum. Similarly to the $s(k) = f(k)$ function, the break on this curve appears at $k = 5$, and therefore this k can be taken as evidence that four complex species are responsible for the absorbance matrix. Table 1 shows the results for the INDICES output of PAR complex-forming systems with four various metal ions when the absorbance matrix was measured for n solutions with total concentrations of c_M, c_L, and c_H for a metal ion, ligand, and proton, respectively, and different mole ratios of C_M/C_L= 0.5, 1, and 2 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.

According to results listed in Table 1, the number of species in the mixture of PAR and Zn^{2+} and Cd^{2+} at different mole ratios of C_M/C_L= 1 and 2 is 5 and of C_M/C_L= 0.5 is 6. The number of species in the mixture of PAR with Al^{3+} and Fe^{3+} at mole ratios of 1 and 2 is 6 and of 0.5 is 7.

4.3. Chelating Mode of PAR to the Metal Ions. W. J. Greay et al.[31] reported the mode of chelation of PAR with some metal ions. They concluded the atoms involved in the coordination phenomenon are pyridine nitrogen (or heterocyclic nitrogen), the azo nitrogen farthest from the heterocyclic ring, and the *o*-hydroxyl group. Their observations are clear evidence in favor of chelation involving two five-membered rings rather than one six- and one four-membered ring. Further evidence of the terdentate nature of PAR and the greater part in chelation of the azo nitrogen farthest from the heterocyclic ring may be adduced from the decrease in stability on changing from a 1:1 to a 2:1 complex. Thus, in the terdentate ligand like PAR, the value of $\log K_1 - \log K_2$ is large and almost equal. Further, while $\log K_1 - \log K_2$ is large for metal ions like Cu^{2+} with ligands like PAR, it is smaller for those ions more ready to be six covalent.

4.4. Determination of Stability Constants. Absorbance mole ratio data matrices by applying the procedure of efficient experimentation and computational strategy at $C_M/C_L = 0.5$, 1, and 2 were obtained. The concentrations of Zn^{2+}, Cd^{+2}, Fe^{3+}, and Al^{3+} were $3.0 \cdot 10^{-5}$, $6.0 \cdot 10^{-5}$, and $8.0 \cdot 10^{-5}$ M and $6.0 \cdot 10^{-5}$, $6.0 \cdot 10^{-5}$, and $4.0 \cdot 10^{-5}$ M at ratios 0.5, 1, and 2, respectively. The complexation reaction of the metal ions with PAR as a ligand was supported by visually detected spectral changes, which related to the variation of the solution from a yellow to a red color.

The chemical model concerns the number of light-absorbing species coexisting in the equilibrium mixture, their stoichiometries, and their stability constants which are estimated by regression analysis and, at the same time, the curves of molar absorption coefficients in their dependence on wavelength. For a set of current values of β_{pqr}, the free concentrations of the metal [M], the ligand [L], and [H] (known from pH measurements) for each solution are calculated, and then the concentrations of all the complexes in the equilibrium mixture, $[M_pL_qH_r]_j$, $j = 1, ..., n_c$, forming for n solutions the matrix **C** are found. In

Table 2. Log β_{pqr}, Sum of Squares (RSS), and Standard Deviation ($s(\log \beta_{pqr})$) of the Most Probable Chemical Model in the PAR Complex-Forming System with Zn^{2+}, Cd^{2+}, and Al^{3+} Ions and Fe^{3+} Estimated by EQUISPEC and SQUAD Programs[a]

	$C_M/C_L = 0.5$		$C_M/C_L = 1$		$C_M/C_L = 2$		simultaneous analysis using EQUISPEC	
species	log β_{pqr}	RSS	log β_{pqr}	RSS	log β_{pqr}	RSS	log β_{pqr}	RSS
ZnL[b]	**10.50(0.15)**[c]	**0.25**	**10.64(0.05)**	**0.03**	**10.30(0.16)**	**0.22**		
ZnLH	**16.97(0.20)**	**0.25**	**16.86(0.04)**		**16.24(0.25)**			
ZnL	10.72(0.05)	0.007	10.69(0.02)	0.01	11.00(0.09)	0.02	10.89(0.21)	0.17
ZnLH	17.23(0.06)	0.007	17.02(0.02)		17.10(0.09)		17.12(0.21)	
CdL	**9.29(0.05)**	**0.009**	**9.65(0.03)**	**0.006**	**9.76(0.06)**	0.004		
CdLH	**16.15(0.09)**	**0.009**	**16.52(0.04)**		**16.48(0.13)**			
CdL	9.23(0.01)	0.007	9.78(0.02)	0.007	9.05(0.02)	0.004	9.45(0.13)	0.09
CdLH	16.23(0.03)	0.007	16.69(0.02)		16.83(0.03)		16.41(0.18)	
AlLH	**14.95(0.10)**	**0.10**	**14.91(0.03)**	**0.02**	**15.20(0.04)**	**0.10**		
AlL_2H	**24.72(0.06)**	**0.11**	−−		−−			
AlLH	15.01(0.04)	0.02	15.75(0.02)	0.07	15.890(0.03)	0.12	15.80(0.24)	0.12
AlL_2H	24.81(0.08)	0.02	−−		−−		25.00(0.41)	
FeLH	**16.05(0.11)**	**0.02**	**16.16(0.08)**	**0.01**	**16.11(0.09)**	**0.01**		
FeL_2H	**24.31(0.12)**	**0.02**	−−		−−			
FeLH	15.90(0.04)	0.02	16.04(0.04)	0.01	15.87(0.04)	0.01	15.98(0.18)	0.15
FeL_2H	24.23(0.04)	0.02	−−		−−		24.62(0.54)	

[a] The selected analytical pH for Al^{3+} and Fe^{3+} is smaller than 6. The simultaneous analysis using EQUISPEC used the data sets for all three metal/ligand ratios. [b] Boldcase determined by SQUAD. [c] Values in parentheses are $s(\log \beta_{pqr})$.

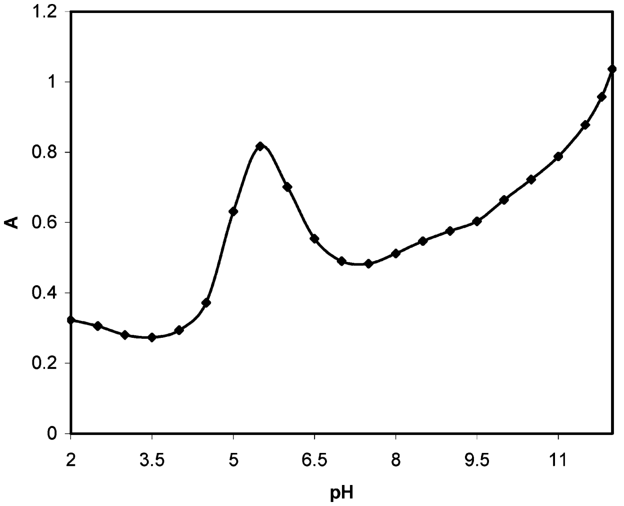

Figure 7. Change of absorbances vs pH at $\lambda = 499$ nm for PAR−Al^{3+} at $C_M/C_L = 1.0$.

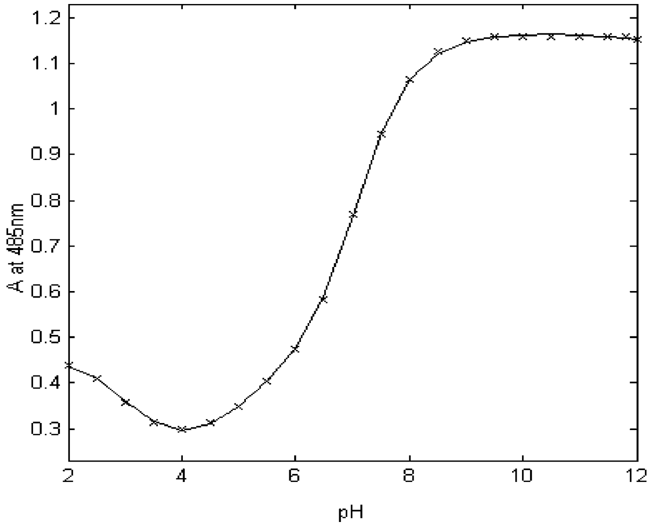

Figure 8. Curve fit of titration of PAR with Cd^{2+} in $C_M/C_L = 1$.

addition to the fit achieved, it is also necessary to examine the physicochemical sense of the model parameter 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 %(minor species), the evaluation of such an equilibrium can fail; i.e., from the spectral point of view, it acts as “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.

PAR forms complexes ML and MLH (M, metal; and L, ligand) with Zn^{2+} and Cd^{2+}, just as achieved by the EQUISPEC program in the distribution curves (Figures 5 and 6), and some L remained in the free form in the environment when the ligand PAR was in excess (C_M/C_L= 0.5), which caused an increase in the number of species. In accordance with results of the PCA INDICES analysis, the hypothesis of five species, respectively, was also tested, and better fitness is observed for the spectral data points. Therefore, the final chemical model contained five light-absorbing components in the system of PAR with Zn^{2+} and Cd^{2+} in C_M/C_L = 1 and 2 and six light-absorbing components in the system of PAR with Zn^{2+} and Cd^{2+} in C_M/C_L= 0.5. Stability constants calculated for these two species in different mole ratios by the SQUAD and EQUISPEC programs are shown in Table 2.

EQUISPEC has two modes for the calculation of the stability constants of the proposed model, the global and local modes. In the global mode, it considers all titration matrices in the calculation, and in the local mode, each titration matrix is used independently. In Table 2, the last two columns are the resulting stability constants using global analysis of all the titration matrices for all C_M/C_L ratios and the total residual sum of squares. It is not surprising to mention that the global analysis has enough ability to distinguish all expected species formed from the initial components. It means the application of a single titration set such as that at ratio C_M/C_L = 1 and 2 in the case of Fe^{3+} and Al^{3+} results in a misleading chemical equilibrium model.

In the case of Al^{3+} and Fe^{3+}, the hydrolysis of the metal cations caused complicated situations. Sommer and Novotna[32] observed interesting behavior of the Al^{3+}−PAR system. They plotted the absorbance vs pH at selected wavelengths and two C_L/C_M ratios. In this plot, the absorbance vs pH has an increasing pattern until pH = 4.7 and then decreases with the addition of the pH which indicates the decomposition of the metal complexes due to formation of the metal hydroxide species. The same behavior is observed here in the case of Al^{3+}. Figure 7 shows the plot of absorbance−pH data for the Al^{3+}−PAR system, and the trend of increasing and decreasing absorbance values at $\lambda = 499$ nm is easily observed. Then we collected the spectral pH traces under pH 6.0. In the case of Fe^{3+}, the hydrolysis complicated the absorption−pH plots, and then we limited the final pH to under 6.

The distribution curves, results of EQUISPEC, showed that PAR formed different protonated complex species, MLH and ML$_2$H, with the two cations Al^{3+} and Fe^{3+} in the mole ratio $C_M/C_L = 1$ and 2. In accordance with the results of the PCA INDICES analysis, the hypothesis of five and six species, respectively, was also tested, and better fitness through spectral data points was achieved. Therefore, the final chemical model contained five light-absorbing components in the system of PAR with Al^{3+} and Fe^{3+} in $C_M/C_L = 1$ and 2 and six light-absorbing components in the system of PAR with Al^{3+} and Fe^{3+} in $C_M/C_L = 0.5$.

Calculated stability constants of the resulting complexes by given programs are listed in Table 2. A change in the stability constants is accompanied by a change in the Gibbs free energy, so the stability constants of ternary species such as $M_pL_qH_r$ can be determined from the binary parent species. So, as is clear from the data in Table 2, the stability constants of species MLH and MHL$_2$ can be extracted from equilibrium reactions like 4 and 5. The stability constant of LH can be determined from the subtraction of log β_{110} of ML from the log β_{111} value of MLH.

$$\mathrm{M} + \mathrm{LH_3} \rightleftarrows \mathrm{MLH} + 2\mathrm{H}^+ \tag{4}$$

$$\mathrm{MLH} \rightleftarrows \mathrm{ML} + \mathrm{H}^+ \tag{5}$$

The validity of the stability constants is checked by calculation of several statistical parameters. The sum of squared residuals is computed from the difference between calculated and experimental spectra at each titration step. A sample of the fitting process in a selected wavelength is shown in Figure 8. Another criterion is the standard deviation of each stability constant which is commonly estimated from the Hessian matrix implemented in SQUAD and EQUISPEC software. The correlation matrix of each equilibrium system is an indication of the correlation of the unknown parameters. These statistical values are lower than the accepted value of 0.7 for all final equilibrium models of PAR with metal ions.

5. Conclusion

The stability constants of the complexes of PAR with Zn^{2+}, Cd^{2+}, Al^{3+}, and Fe^{3+} in various mole ratios ($C_M/C_L = 0.5$, 1, and 2) were estimated using spectrophotometric titration. Tentative methods which all are implemented in the computer program INDICES are applied for the determination of the light-absorbing species. The versatility of the global analysis is employed for detection of the minor species in equilibrium systems by using the program EQUISPEC.

Literature Cited

(1) Ohyoshi, E. Relative stabilities of metal complexes of 4-(2-pyridylazo)-resorcinol and 4-(2-thiazolylazo)resorcinol. *Polyhedron* **1986**, *5* (6), 1165−1170.

(2) Malinowski, E. R. *Factor Analysis in Chemistry*, 2nd ed.; Wiley: New York, 1991.

(3) Meloun, M.; Havel, J.; Högfeldt, E. *Computation of Solution Equilibria*; Horwood: Chichester, 1988.

(4) Malinowski, E. R. Abstract factor analysis of data with multiple sources of error and a modified Faber−Kowalski F-test. *J. Chemom.* **1999**, *13*, 69−81.

(5) Malinowski, E. R. Determination of the number of factors and the experimental error in a data matrix. *Anal. Chem.* **1977**, *49* (4), 612−617.

(6) Elbergali, A. K.; Nygren, J.; Kubista, M. An automated procedure to predict the number of components in spectroscopic data. *Anal. Chim. Acta* **1999**, *379* (1), 143−158.

(7) Dean, J. M. In *Multivariate Pattern Recognition in Chemometrics Illustrated by Case Studies*; Brereton, R. G., Ed.; Elsevier: Amesterdam, 1992.

(8) Meloun, M.; Čapek, J.; Mikšík, P.; Brereton, R. G. Critical comparison of methods predicting the number of components in spectroscopic data. *Anal. Chim. Acta* **2000**, *423* (1), 51−68.

(9) Elbergali, A. K.; Nygren, J.; Kubista, M. An automated procedure to predict the number of components in spectroscopic data. *Anal. Chim. Acta* **1999**, *379* (1), 143−158.

(10) Rossi, T. M.; Warner, I. M. Rank estimation of excitation−emission matrices using frequency analysis of eigenvectors. *Anal. Chem.* **1986**, *58* (4), 810−815.

(11) Kankare, J. J. Computation of equilibrium constants for multicomponent systems from spectrophotometric data. *Anal. Chem.* **1970**, *42* (12), 1322−1326.

(12) Catell, R. D. The Scree. Test for the Number of Factors. *Multi. Behav. Res.* **1966**, *1*, 245.

(13) (a) Leggett, D. J., Ed. *Computational Methods for the Determination of Formation Constants*; Plenum Press: New York, 1985; pp 99−157. (b) Leggett, D. J., Ed. *Computational Methods for the Determination of Formation Constants*; Plenum Press: New York, 1985; pp 291−353. (c) Havel, J.; Meloun, M.; Leggett, D. J., Eds. *Computational Methods for the Determination of Formation Constants*; Plenum Press: New York, 1985; p 19. (d) Havel, J.; Meloun, M.; Leggett, D. J., Eds. *Computational Methods for the Determination of Formation Constants*; Plenum Press: New York, 1985; p 221.

(14) Leggett, D. J.; McBryde, W. A. E. General computer program for the computation of stability constants from absorbance data. *Anal. Chem.* **1975**, *47* (7), 1065−1070.

(15) Meloun, M.; Javůrek, M.; Havel, J. Multiparametric curve fitting−X: A structural classification of programs for analysing multicomponent spectra and their use in equilibrium-model determination. *Talanta* **1986**, *33* (6), 513−524 (or on internet http://meloun.upce.cz).

(16) Dyson, R. M.; Kaderli, S.; Lawerance, G. A.; Maeder, M.; Zuberbühler, A. D. Second order global analysis: the evaluation of series of spectrophotometric titrations for improved determination of equilibrium constants. *Anal. Chim. Acta* **1997**, *353* (2), 381−393.

(17) Leggett, D. J. Numerical analysis of multicomponent spectra. *Anal. Chem.* **1977**, *49* (2), 276−281.

(18) Meloun, M.; Militký, J.; Forina, M. *Chemometrics for Analytical Chemistry. 2. PC-Aided Regression and Related Methods*; Horwood: Chichester, 1994. Meloun, M.; Militký, J.; Forina, M. *Chemometrics for Analytical Chemistry. 1. PC-Aided Statistical Data Analysis*; Horwood: Chichester, 1992.

(19) Martell, A. E.; Motekaities, R. J. *Determination and Use of Stability Constants*; Verlag Chemie: Weinheim, 1988.

(20) Polster, J.; Lachmann, H. *Spectrometric Titration*; Verlag Chemie: Weinheim, 1989.

(21) Bevington, P. R. *Data Reduction and Error Analysis for the Physical Sciences*; McGraw-Hill: New York, 1969.

(22) Golub, G. H.; Van Loan, C. F.; *Matrix Computations*, 2nd ed.; John Hopkins University Press: Baltimore, 1989.

(23) Bugnon, P.; Cottared, J. C.; Jestin, J. L.; Bung, B.; Laurenczy, G.; Maeder, M.; Merbach, A. E.; Zuberbühler, A. D. Second-order globalisation for the determination of activation parameters in kinetics. *Anal. Chim. Acta* **1994**, *298* (2), 193−201.

(24) Knutson, J. R.; Beechem, J. M.; Brand, L. Simultaneous analysis of multiple fluorescence decay curves: A global approach. *Chem. Phys. Lett.* **1983**, *102* (6), 501−507.

(25) Beechem, J. M. Global analysis of biochemical and biophysical data. *Methods Enzymol.* **1992**, *210*, 37.

(26) Janssens, L. D.; Bones, N.; Ameloot, M.; De Schryver, F. C. A systematic study of the global analysis of multlexponential fluorescence decay surfaces using reference convolution. *J. Phys. Chem.* **1990**, *94* (9), 3564−3576.

(27) Beechem, J. M.; Hass, E. Simultaneous determination of intramolecular distance distributions and conformational dynamics by global analysis of energy transfer measurements. *Biophys. J.* **1989**, *55*, 1225.

(28) Braibanti, A.; Ostaroli, G.; Paoleti, P.; Pettit, D.; Sammartano, S. Recommended procedure for testing the potentiometric apparatus and technique for the pH-metric measurement of metal-complex equilibrium constants. *Pure Appl. Chem.* **1987**, *59*, 1721.

(29) Metzler, D. E. *Biochemistry: The Chemical Reaction of Living Cells*, 2nd ed.; Harcourt/Academic Press: San Diego, 2001.

(30) May, P. M.; Williams, D. R.; Linder, P. W.; Torrington, R. G. The use of glass electrodes for the determination of formation constants—I. A definitive method for calibration. *Talanta* **1982**, *29* (4), 249–256.
(31) Greay, W. J.; Nickless, G.; Pollard, F. H. The metal complexes of some azo and azomethine dyestuffs. Part II. Determination of stability constants and preparation of solid complexes. *Anal. Chim. Acta* **1962**, *27*, 71–79.
(32) Sommer, L.; Novotna, H. Complexation of aluminum, yttrium, lanthanum and lanthanides with 4-(2-pyridylazo)resorcinol (PAR). *Talanta* **1967**, *14*, 457–471.

Received for review July 20, 2006. Accepted April 14, 2007.

JE060325G