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Rank annihilation factor analysis for spectrophotometric study of complex formation equilibria

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Abstract

The use rank annihilation factor analysis (RAFA) for spectrophotometric studies of complex formation equilibria are proposed. One-step complex formation and two successive and mononuclear complex formation systems studied successfully by proposed methods. When the complex stability constant acts as an optimizing object, and simply combined with the pure spectrum of ligand, the rank of original data matrix can be reduced by one by annihilating the information of the ligand from the original data matrix. The residual standard deviation (R.S.D.) of the residual matrix after bilinearization of the background matrix is regarded as the evaluation function. The performance of the method has been evaluated by using synthetic data. For two-step successive complex formation systems, the effects of noise level and equilibrium constants K_1 and K_2 on output of algorithm are investigated. The applicability of method for resolving the two-step successive complex formation systems with full spectral overlapping of two complex species also is shown. Spectrophotometric studies of murexide–calcium, dithiazone–nickel and methyl thymol blue (MTB)–copper are used as experimental model systems with different complexation stoichiometries and spectral overlapping of involved components.

Keywords: Complex formation equilibria; RAFA; Mole ratio method; Two-way spectral data

1. Introduction

Equilibrium data provide important means for speculation about the structural parameters influencing stability, linearity of free energy relations and solvent effects. Stability constants are very important both in the analysis of drugs, as well as, in the interpretation of their mechanism of action [1]. So up to present, a large number of papers have been devoted to the study of complexation reaction between metal ions and various ligands [2–4]. In spite of the large number of papers published, many gaps remain. A further significant defect is that some of the data are not reliable. Precise method should be applied to obtain reliable data on the complexation reactions. Among the several physicochemical methods for studying the complexation equilibria in solution, spectrophotometry (i.e. UV-Vis) under broad experimental conditions and with subsequent computer treatment of experimental data is a very powerful method [5]. Spectrophotometric methods are in general highly sensitive and as such are suitable for studying chemical equilibria in solution. When the components involved in the chemical equilibrium have distinct spectral responses, their concentrations can be measured directly, and the determination of equilibrium constant is trivial. However, in many cases, the spectral responses of two and sometimes even more components overlap

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considerably and analysis is no longer straightforward. Several spectrophotometric methods have been developed to determine the equilibrium constants of chemical processes. Occasionally, problems arise because of strong overlapping of chemical components involved in equilibrium and some uncertainties from using some complex mathematical algorithms, to solve such problems [6–8].

Several univariate least squares curve fitting methods have been traditionally proposed to interpret the experimental data [9,10] and they have shown, its power to determine the stoichiometry and the formation constants. However, much more information can be extracted if multivariate spectroscopic data are analyzed by means of an appropriate multivariate data analysis method. On the other hand, equilibrium transformations, especially complexation ones, sometimes involve minor changes in their electronic spectra, which make it impossible to employ classical spectrophotometry for determination of equilibrium constants [11].

Modern analytical chemistry has benefited from the development of second- and higher-order instrumentation, that is, instruments capable of providing bi- and multidimensional data arrays, which usually results in a substantial improvement of the analytical capability [12]. In the last two decades, second-order instruments and techniques have become commonplace in analytical chemistry laboratories. Second-order techniques rely on two separated analytical mechanisms linked in series such that the signal of the later is modulated by former. A second-order tensor or a data matrix is generated for each single measurement run. This is the case for the so-called hyphenated techniques such as LC/UV-Vis, LS/MS, GC/MS, GC/FTIR, MS/MS and 2D-NMR [13,14], and other techniques such as two-dimensional excitation–emission fluorometry [13,14], multichannel detection spectroscopic titration [15], kinetic diode array spectroscopic processes [16] and flow optical sensors [17,18].

The spectra measured from a chemical equilibrium process can form a second-order data matrix which contains both the equilibrium information and the pure spectrum of each component involved in the chemical process. The proper chemometric algorithms can be used for evaluating the equilibrium information such as the stability constant through analysis of the second-order spectroscopic data. Several soft- and hard-modeling algorithms have been developed that analyze bilinear data obtained from chemical systems. Examples include kinetics, equilibrium studies and chromatography [19–22]. Soft-modeling methods range from very general approaches with minimal demands on the structure of data, such as EFA [23], HELP [24], SIMPLISMA [25] and ALS [26], to methods which rely on trilinearity, such as PARAFAC [27], GRAM [28] or TLD [29]. Hard-modeling approaches of fitting multivariate response data are based on mathematical relationships, which describe the measurements quantitatively [30,31]. In chemical equilibria, the analysis is based on the equilibrium model which quantitatively describes the reaction and all concentrations in the solution under investigation.

Recently a mixed approach including hard- and soft-modeling has been developed [32,33]. There is a mutual benefit in the use of such mixed methodology. Hard–soft-modeling equals or overcomes the performance of hard- and soft-modeling in any case. The potential usefulness of the novel methodology in kinetics and equilibrium studies is reported by some researchers [19,32,34].

Rank annihilation factor analysis (RAFA) is an efficient chemometric technique based on rank analysis for two-way spectrum data and can be employed to analyze the gray system with unknown background quantitatively. RAFA is comparable with hard–soft-modeling approach in solving some chemical problems. RAFA was originally developed by Ho et al. as an iterative procedure [35]. It was modified by Lorber to yield a direct solution of a standard eigenvalue problem [36,37]. Sanchez and Kowalski extended the method to the general case of several components that are not necessarily present in both the calibration and the unknown samples, obtained the solution by solving a generalized eigenproblem and called the method generalized rank annihilation method (GRAM) [38]. RAFA is used to analyze the two-way chromatographic spectral data [39], for determination environmental pollutants such as polyring aromatic compounds [40,41] and recently for spectrophotometric study of chemical kinetics [42] and the correction of several types of instrumental inconsistencies [43].

RAFA, as shown in this work, can be used for resolving a chemical system which suffers from the one type of rank deficiency problem. A data matrix is rank

deficient when the number of significant contribution to the data variance, estimated by using singular value decomposition or other related factor analysis techniques, is lower than the real number of chemical components present in the system [44]. The RAFA algorithm can solve the rank deficiency arise from the full spectral overlapping of some chemical species involved in chemical process. The rank deficient matrix can be resolved without combining the other matrices with suitable information (multiple process run [32,44,45]) to reveal the presence of hidden components.

To second-order data matrix measured in chemical complexation reactions, the pure spectrum of each reactant can be reached while that of the intermediate usually remain unknown and the concentration of each species in the equilibrium process is not directly available, but they do change conforming to certain complexation equilibria model. The resolving of such kind problem is similar to gray system with unknown background, where RAFA can be successfully adopted. To the best of our knowledge this is the first application of RAFA for Spectrophotometric studies of complexation equilibria. Although the method has been used successfully for quantitative analysis, kinetic study [42] and correction of instrumental inconsistencies [43], the feasible abilities of RAFA for studying complexation equilibria are not considered so far. The principles for the determination of the stepwise conditional formation constants and spectral profile of each component are deduced through a two-step successive complexation reaction model. The method can be used for spectrophotometric study of one-step complex formation with known stoichiometry. In this work, both numerically simulated and real spectral data were processed.

2. Experimental

UV-Vis absorbance digitized spectra were collected using a CARY5 spectrophotometer, 1 cm quartz cells, a scan rate of 100 nm/min and a slit width of 2 mm. The recorded spectra were digitized with one data point per nanometer. Measurements of pH were made with Metrohm 713 pH-meter using a combined glass electrode. Murexide, dithiazone, methyl thymol blue (MTB) and all metal ion salts were purchased from Merck and used without further purification. All of the solutions were prepared fresh daily. Solutions were allowed to remain in a thermostated sample compartment for minimum of 10 min before the spectra were collected. The temperature was maintained at 25.0 ± 0.1 °C using a Fisher Scientific Isotemp constant temperature circulator. Specific details are given in Section 3. All calculations are performed in MATLAB 6.0 (Math Works, Cochituate Place, MA).

3. Results and discussion

3.1. Theoretical background

The two successive stepwise conditional complex formations are defined by chemical equilibria

$$M + L \rightleftarrows ML \quad (1)$$

$$ML + L \rightleftarrows ML_2 \quad (2)$$

The corresponding stepwise complex formation constants are

$$K_1 = \frac{[ML]}{[M][L]} \quad (3)$$

$$K_2 = \frac{[ML_2]}{[ML][L]} \quad (4)$$

The mass balances of the system in different mole ratios of metal–ligand can be written as

$$C_L = [L] + [ML] + 2[ML_2] \quad (5)$$

$$C_M = [M] + [ML] + [ML_2] \quad (6)$$

At mole ratio equal to zero the recorded spectrum is the pure spectrum of C_L concentration of ligand. So, in the spectral region that ligand and two complexes have strong spectral overlapping, each solution at a particular mole ratio can be considered as a mixture solution of three components with known absorption spectrum of ligand component.

The combination of Eqs. (3)–(6) as a function of the free ligand concentration [L] yields the expression

$$K_1K_2[L]^3 + (K_1(1 + K_2(2C_M - C_L)))[L]^2 + (1 + K_1(C_M - C_L))[L] - C_L = 0 \quad (7)$$

The concentration profile of free ligand can be calculated for certain K_1 and K_2 values by obtaining the

roots of this equation as a function of C_M. The equilibrium concentration of ligand at different mole ratio can formed a column vector [L] and it is referred to as the concentration profile of the ligand species. Assume that ligand and complex species are absorptive in the measured wavelength range and in comparing with most real systems the metal ion species is not considered as an absorbing component. A two-way data matrix A with rank 3 can be formed by measuring absorbance under different wavelengths at a series of chosen mole ratios

$$\begin{aligned} A &= A_L + A_{ML} + A_{ML_2} + R = \varepsilon_L[L]^T \\ &\quad + \varepsilon_{ML}[ML]^T + \varepsilon_{ML_2}[ML_2]^T + R \\ &= EC^T + R \end{aligned} \tag{8}$$

where A_L, A_{ML} and A_{ML_2} are the bilinear measuring matrix of pure ligand, 1:1 and 1:2 species, respectively and each one can be decompose into the corresponding molar absorptivity spectrum ε_{ML}, ε_{ML} and ε_{ML_2} (column vectors) and the concentration profiles $[L]^T$, $[ML]^T$ and $[ML_2]^T$ (row vectors, superscript T denotes the transpose of a matrix or vector). E and C^T represent matrices formed, respectively by the molar absorptivity spectrum and the concentration profile of each species. R is the residual matrix and should contain only noise. The size of matrix A is $w \times c$, where w denotes the number of wavelengths and c the number of mole ratios for which absorbances were recorded (c is smaller than w). Obviously, the size of matrix E and C^T are $w \times 3$ and $3 \times c$, respectively.

For the two successive and mononuclear complex formation system, represented in Eqs. (1) and (2), when the mole ratio method is used, the pure ligand spectrum can be readily measured at mole ratio equal to zero, while the pure spectra of intermediate complex (ML_2 species) is usually unknown. The primary purpose of investigating the considered complexation model is to acquire the pure spectra of each species, and equilibrium parameters. Let

$$F = A - A_L = A - \varepsilon_L[L]^T \tag{9}$$

The aim of RAFA approach is to find a suitable set of K_1 and K_2 so that the rank of matrix F can be reduced by 1 from that of matrix A through introduction of the concentration profiles of ligand species obtained from the roots of the Eq. (7).

Upon specific operations the grid searching method can optimize the set of K_1 and K_2. The optimized solutions can be reached by decomposing matrix F to the extent that the residual standard deviation (R.S.D.) [46] of the residual matrix obtained after the extraction of two principal components reaches the minimum. Under condition that the two stability constants, K_1 and K_2, or the concentration profile of three species, are available, the pure absorption spectrum of each component can be reached by means of least square regression.

$$E = AC(C^TC)^{-1} \tag{10}$$

3.2. Simulation

To evaluate the performance of the method, several sets of synthetic data were created according to mole ratio method for studying the various complex formation systems. The use of simulated data allows us to know how each problem affects the performance of the considered method. For simulating the two successive mononuclear complexation systems, three spectra were created and summed together in known proportion chosen to mimic model of mole ratio method. Simulated spectra of species were produced by Gaussian function. Random error was added to the set of artificial data generated to more rigorously test the method. The error is a set of noise in agreement with the Gaussian distribution with mean zero and standard deviation equal to 0.2% of absorbance value. A model based on mole ratio method was used to calculate the concentration profiles of ligand and both complex species and thus weight the contribution of each pure spectrum in the total spectrum generated using a multicomponent Beer's law expression. The total concentration of ligand was kept constant in the creation of the synthesized spectral data since our experimental data are collected under this condition. The polynomial equation (Eq. (7)) which results from the combination of the formation constant and the mass balance for the metal and the ligand can be used for calculating the ligand equilibrium concentration profile.

If the values of C_M, C_L, K_1 and K_2 are known, it is possible to obtain the free ligand concentration [L] from the roots of the associated polynomial. This can be done numerically by the command *roots* of MATLAB. It then becomes a question of selecting

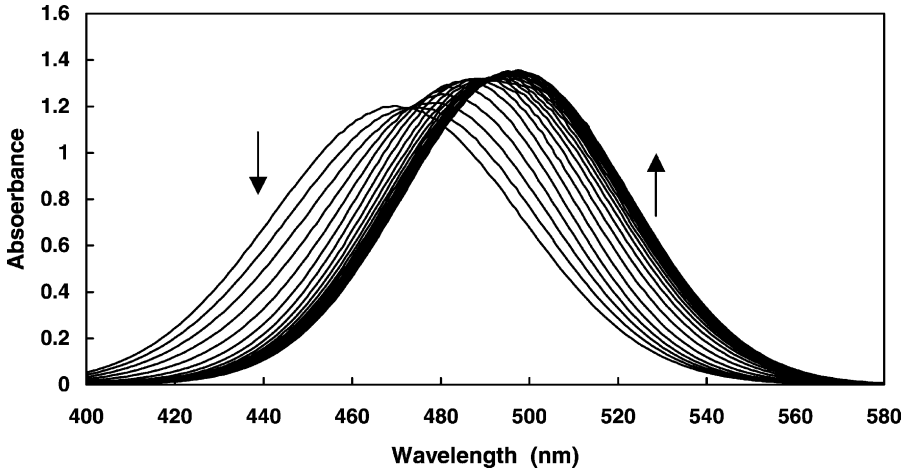

Fig. 1. Simulated absorption spectra of a hypothetical two successive steps 1:1 and 1:2 metal–ligand complex formation systems at the fixed concentration of ligand and various concentrations of metal ions. $\log(K_1) = 5.00$, $\log(K_2) = 4.20$, $\lambda_{max}(L) = 450$ nm, $\lambda_{max}(ML_2) = 465$ nm, $\lambda_{max}(ML) = 480$ nm.

the proper value of [L] from the non-complex and non-negative roots (usually it is the minimum one). The equilibrium concentration of remaining species can be easily computed from the combination of Eqs. (3)–(6). In creating the data sets the $\log(K_1) = 5.00$ and $\log(K_2) = 4.20$ were used. The generated spectra corresponded to mole ratio ranging from 0 to 2.

The conditional complex formation between metal ion, M, and ligand, L in one step also is considered and simulated. The related chemical equilibrium is

$$M + nL \rightleftarrows ML_n \tag{11}$$

The corresponding complex formation constant, K_f, is

$$K_f = \frac{[ML_n]}{[M][L]^n} \tag{12}$$

where [L], [M] and $[ML_n]$ are the equilibrium concentrations of ligand, metal ion and the complex, respectively. The mass balances of the system in different mole ratio of metal–ligand can be written as

$$C_L = [L] + n[ML_n] \tag{13}$$

$$C_M = [M] + [ML_n] \tag{14}$$

where C_L is the total concentration of ligand, which remains constant, and C_M the total concentration of metal ion, which varies in employing of the mole ratio method. At zero mole ratio, the recorded spectrum is the pure spectrum of C_L concentration of ligand. It is clear that n can be determined according to mole ratio plot [47] or applying continuous variation method [48]. The combination of Eqs. (12)–(14) as a function

Table 1
The results of PCA on simulated data

i	g_i			g_i/g_{i+1}			R.S.D.		
	1:1 and 1:2	1:2	1:1	1:1 and 1:2	1:2	1:1	1:1 and 1:2	1:2	1:1
1	568.96	1437.56	2113.92	23.379	66.418	52.493	3.478	0.541	1.007
2	67.11	21.64	40.27	27.584	54423.95	70955.20	0.0609	0.00007	0.00010
3	2.43	0.00040	0.00057	3823.536	1.039	1.099	0.00009	0.00004	0.00006
4	0.00063	0.00038	0.00052	1.115	1.076	1.031	0.00006	0.00003	0.00004
5	0.00057	0.00036	0.00050	1.156	1.224	1.154	0.00004	0.00002	0.00003
6	0.000494	0.00029	0.00043	1.103	1.020	1.064	0.00003	0.00002	0.00002
7	0.000447	0.00028	0.00041						

of the free ligand concentration, [L], yields the expression

$$K_f[L]^{n+1} + (nK_fC_M - K_fC_L)[L]_n + [L] - C_L = 0 \quad (15)$$

If the values of n, C_M, C_L and K_f are known, it is possible to obtain the free ligand concentration [L] from the roots of the associated polynomial. This can be done also numerically by the command *roots* of MATLAB. Once [L] is known, the equilibrium concentration of remaining species can be easily computed from the combination of mass balance equations, which yields

$$[M] = \frac{C_M}{1 + K_f[L]^n} \quad (16)$$

$$[ML_n] = \frac{K_fC_M[L]^n}{1 + K_f[L]^n} \quad (17)$$

The model data sets are based on $n = 1$ and 2 being studied. It has done so simply to make the data set related to a real system. However, the method is applicable to any one-step complexation system for which n is known. In creating the data sets the $\log(K_f) = 4.50$ (for $n = 1$) and $\log(K_f) = 7.50$ (for $n = 2$) were used. The generated spectra corresponded to mole ratio ranging from 0 to 2. Additionally, after the spectra were summed together using a Beer's law multicomponent expression and mole ratio model, random noise of ±0.2% absorbance unit was added to each spectrum.

3.3. RAFA for simulated and real data

3.3.1. Two successive steps complex formation

Fig. 1 shows created absorption spectra of 1:1 and 1:2 metal–ligand stepwise complex formation system, at the fixed concentration of ligand and various concentrations of metal ion. Based on principal component analysis (PCA), the R.S.D. method is widely used to determine the number of principal components [46,49]. The R.S.D. is a measure of the lack of fit of a principal component model to a data set. The R.S.D. is defined as

$$\text{R.S.D.}(n) = \left(\frac{\sum_{i=n+1}^{c} g_i}{n(c-1)}\right)^{1/2} \quad (18)$$

where g_i is the eigenvalue and n the number of considered principal components. Table 1 presents the eigenvalues, ratios of consecutive eigenvalues and R.S.D. of matrix A created for simulation data of two stepwise complex formation reaction. It should be noted that the ratio of consecutive eigenvalues when $n = 3$ is the maximum at the same time, it also shows that there exist three absorptive components in the system, which is coincide with the assumption of the simulative experiment.

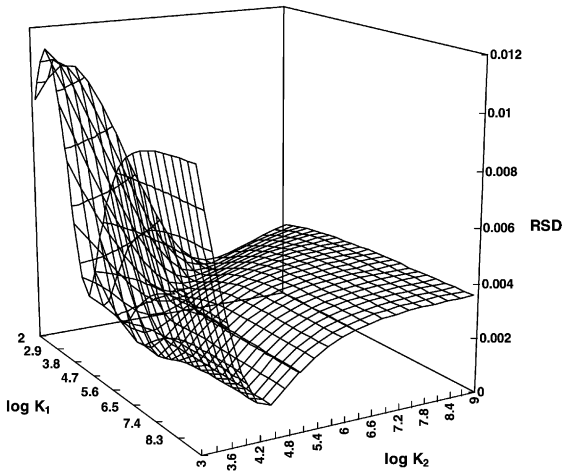

Fig. 2. R.S.D.(2) surface for determination of optimum formation constants for synthetic data of 1:1 and 1:2 complex formation system.

The simulated data matrix A is processed by RAFA method and the relationship between R.S.D.(2) of F matrix and the values of formation constants, K_1 and K_2, is shown as R.S.D. surface in Fig. 2. For each value of K_1, concentration profiles of ligand species for all values of K_2 are calculated and the corresponding F matrices are obtained. Narrow minimum is observed in R.S.D. surface shown in Fig. 2 which indicates the optimum values of K_1 and K_2. For checking the abilities of proposed method, the algorithm was tested by using the simulated data set as a function of the magnitudes of noise level included in the analysis and the conditional formation constant values. The results are summarized in Table 2. The solved and known stability constants are in good accordance as well. It can be resulted that the calculated formation constants are within random variations of actual formation constants used to create the data nearly independent of the magnitude of the formation constants and reliable noise level.

Table 2
Calculated conditional stability constants based on model data as a function of the input formation constant and standard deviation of applied noise

Noise level	$\log(K_1) = 5.00$	$\log(K_2) = 3.80$	$\log(K_1) = 5.00$	$\log(K_2) = 4.20$	$\log(K_1) = 5.50$	$\log(K_2) = 4.80$	$\log(K_1) = 5.50$	$\log(K_2) = 5.00$	$\log(K_1) = 6.00$	$\log(K_2) = 5.50$	$\log(K_1) = 4.50$	$\log(K_2) = 4.00$
0.001	5.00	3.80	5.00	4.20	5.47	4.79	5.49	5.00	5.87	5.50	4.51	4.00
0.002	5.00	3.80	5.01	4.20	5.53	4.79	5.42	5.00	5.92	5.49	4.49	4.00
0.003	4.96	3.77	5.01	4.21	5.51	4.80	5.42	4.99	5.89	5.51	4.51	4.01
0.004	5.01	3.81	5.02	4.19	5.48	4.78	5.49	5.00	5.78	5.51	4.46	3.97
0.005	5.01	3.82	4.98	4.20	5.49	4.82	5.49	4.95	5.76	5.52	4.49	4.01

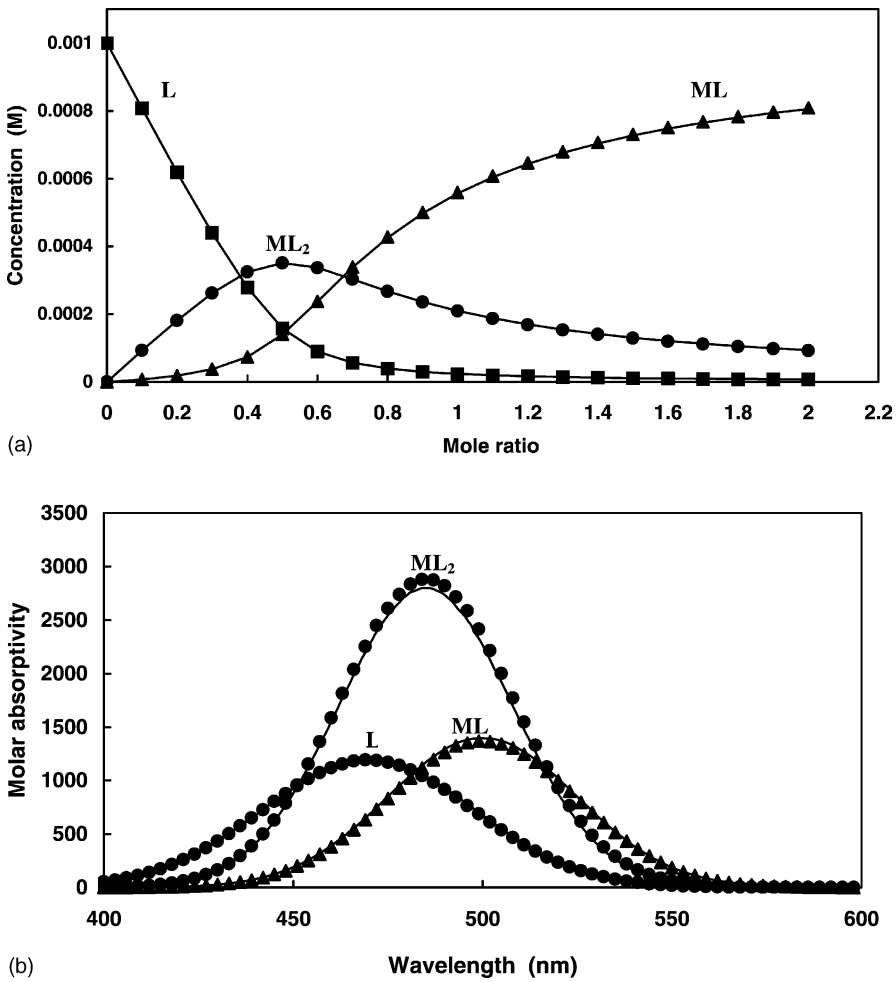

Fig. 3. Results of applying RAFA on synthetic data of 1:1 and 1:2 complex formation system: (a) concentration profiles; (b) absorption spectra.

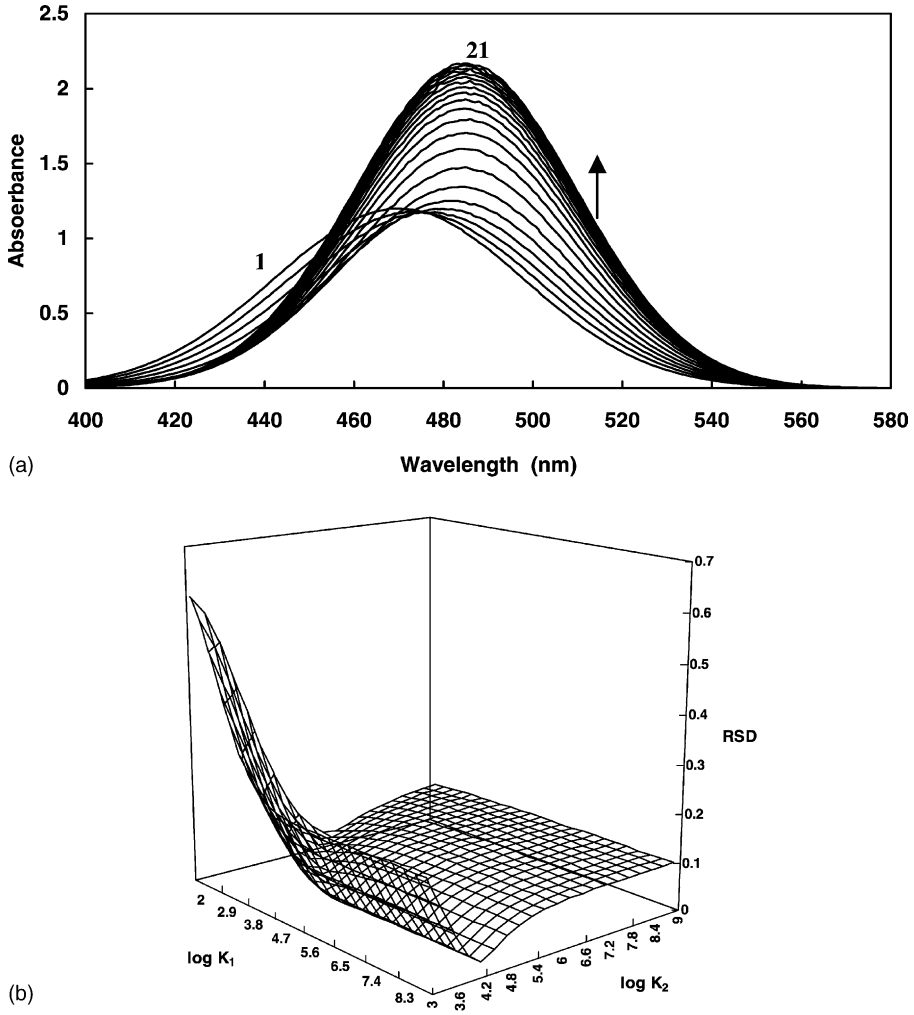

Fig. 4. (a) Simulated absorption spectra of the considered two successive steps 1:1 and 1:2 metal–ligand complex formation systems with full spectral overlapping of ML and ML_2 components, $\log(K_1) = 5.00$, $\log(K_2) = 4.20$, $\lambda_{max}(L) = 450\,nm$, $\lambda_{max}(ML) = \lambda_{max}(ML_2) = 465\,nm$; (b) corresponding R.S.D.(1) surface for determination of optimum formation constants.

The concentration profiles [L], [ML] and [ML_2] can be calculated from resolved and given formation constants, respectively. As shown in Fig. 3a the data point in the figure denote concentration profiles calculated from obtained K_1 and K_2 while the lines present that of given values. It is apparent that the two data sets are in good agreement. The molar absorptivity spectra of each species, which are calculated based on Eq. (10) are illustrated in Fig. 3b. The solved spectra (data mark) and the known spectra (lines) are good accordance as well. From above results, it can be concluded that the RAFA method can be successfully resolve the spectrum of each pure chemical components and equilibrium stability parameters, as well.

The proposed RAFA method can be used for analysis of the mentioned system when the one of the complex species (ML or ML_2) has not absorbance in the monitoring region or even where the spectra of two species are exactly similar. Simulated absorption spectra of a hypothetical two successive steps 1:1 and 1:2 metal–ligand complex formation systems were

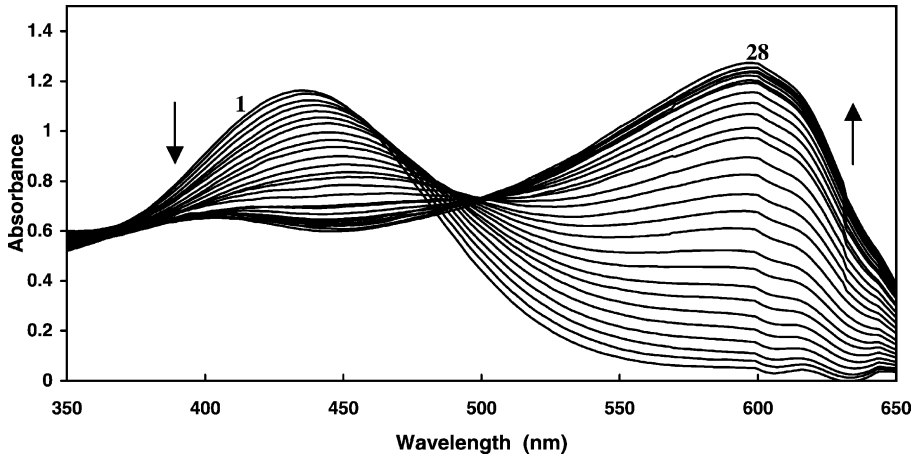

Fig. 5. Experimental absorption spectra of a series of solution containing a constant concentration (1.6×10^{-4} M) of the methyl thymol blue (MTB) at the fixed pH of 5.0 and varying amounts of the Cu^{2+}.

Table 3
The results of PCA on experimental data

i	g_i			g_i/g_{i+1}			R.S.D.		
	MTB–Cu	DT–Ni	Mu–Ca	MTB–Cu	DT–Ni	Mu–Ca	MTB–Cu	DT–Ni	Mu–Ca
1	4120.53	286.75	1980.27	10.698	50.770	70.46	14.378	0.629	0.828
2	385.15	5.65	28.104	127.856	462.34	1114.18	0.0563	0.00070	0.00051
3	3.01	0.012	0.025	246.811	61.569	6.707	0.00039	0.000012	0.00010
4	0.0122	0.0002	0.004	2.503	2.130	3.971	0.00018	0.000003	0.00004
5	0.0049	0.0001	0.0009	1.247	10.048	1.291	0.00011	0.000001	0.00003
6	0.0034	0.00001	0.0007	1.611	1.732	1.231	0.00007	0.000001	0.00001
7	0.0024	0.00001	0.0006						

MTB, methyl thymol blue; DT, dithiazone, Mu, murexide.

created at the fixed concentration of ligand and various concentrations of metal ions where components ML or ML_2 have no absorption ($\log(K_1) = 5.00$, $\log(K_2) = 4.20$, $\lambda_{max}(L) = 450$ nm ML component has no absorption, $\lambda_{max}(ML_2) = 465$ nm and ML_2 component has no absorption, $\lambda_{max}(ML_2) = 480$ nm). After the created data matrices have been processed by RAFA, the relationship between R.S.D.(1) and stability constants K_1 and K_2 can be obtained. For both R.S.D.–K surfaces the narrow minimum were observed which indicate the optimum values of K_1 and K_2. The solved and known stability constants are in good accordance as well. More complex problem in spectrophotometric study of stepwise complex formation is full overlapping of the absorption spectra of complex species. This problem is an example of creating the rank deficient matrix and also can be solved

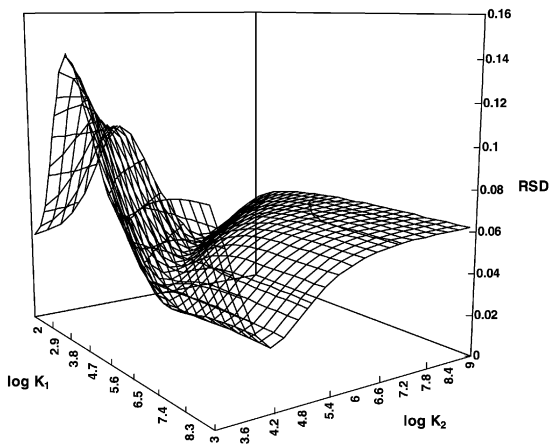

Fig. 6. Relationship between R.S.D.(2) and K values for MTB–Cu system.

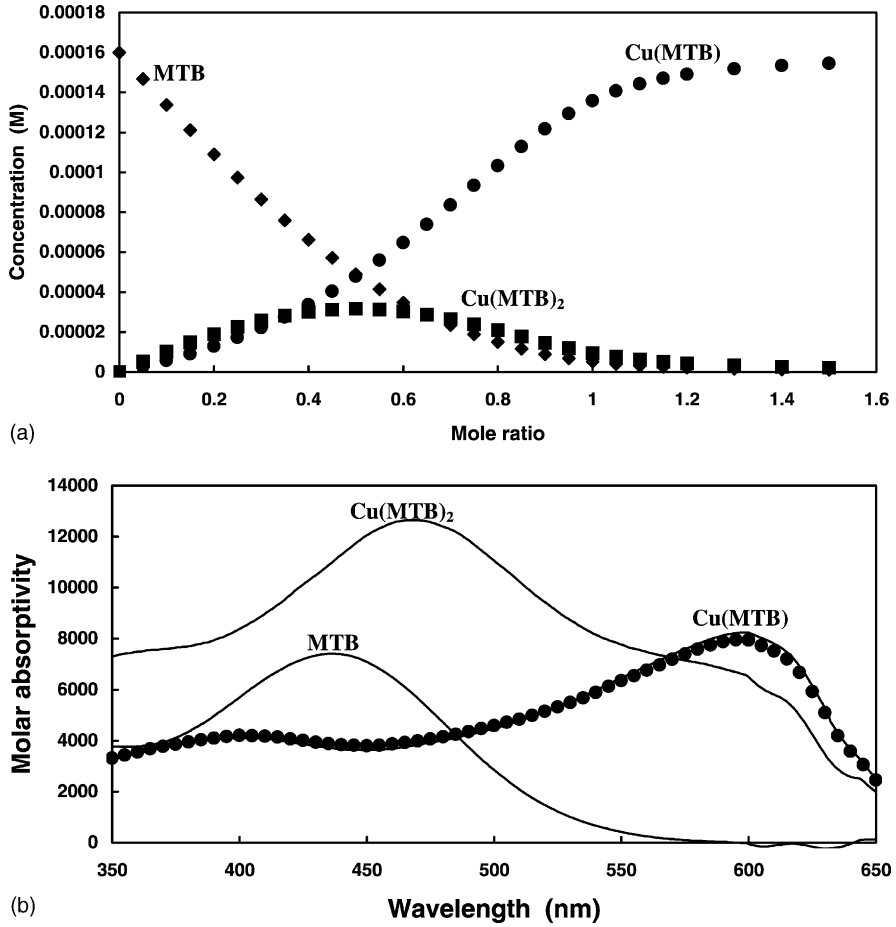

Fig. 7. Results of applying RAFA on experimental data of MTB–Cu complex formation system: (a) concentration profiles; (b) absorption spectra.

by RAFA method. Fig. 4a shows created absorption spectra of the considered system under condition that components ML and ML_2 have the exactly same absorption spectra. The R.S.D.(1)–K surface also is shown in Fig. 4b which indicate the narrow minimum for optimum K_1 and K_2 values. Good accordance exists between solved and known stability constant values.

The favorable results obtained with the artificial test data clearly showed that the approach to computing the equilibrium concentration profiles in two-step successive complex formation is sufficiently accurate for analyzing real spectral data and thus estimating the stepwise formation constants. Fig. 5 shows the absorption spectra of a series of solution containing a constant concentration of the MTB at the fixed pH of 5.0 and varying amounts of the Cu^{2+}. The spectra indicate that Cu^{2+} can form two complexes of 1:1 and 1:2 (metal–ligand) stoichiometry with MTB. While MTB absorbs light at 435 nm, maximum absorption wavelengths for 1:1 and 1:2 complexes are located at about 596 and 499 nm, respectively. In addition, there are two isosbestic points in the corresponding spectra indicating the occurrence of two consecutive equilibria during the evolving process.

The number of absorption spectra also may be determined by applying PCA on data matrix shown in Fig. 5. The results are presented in Table 3. The ratio of consecutive eigenvalues reaches maximum at $i = 3$, therefore indicating there exist three absorptive

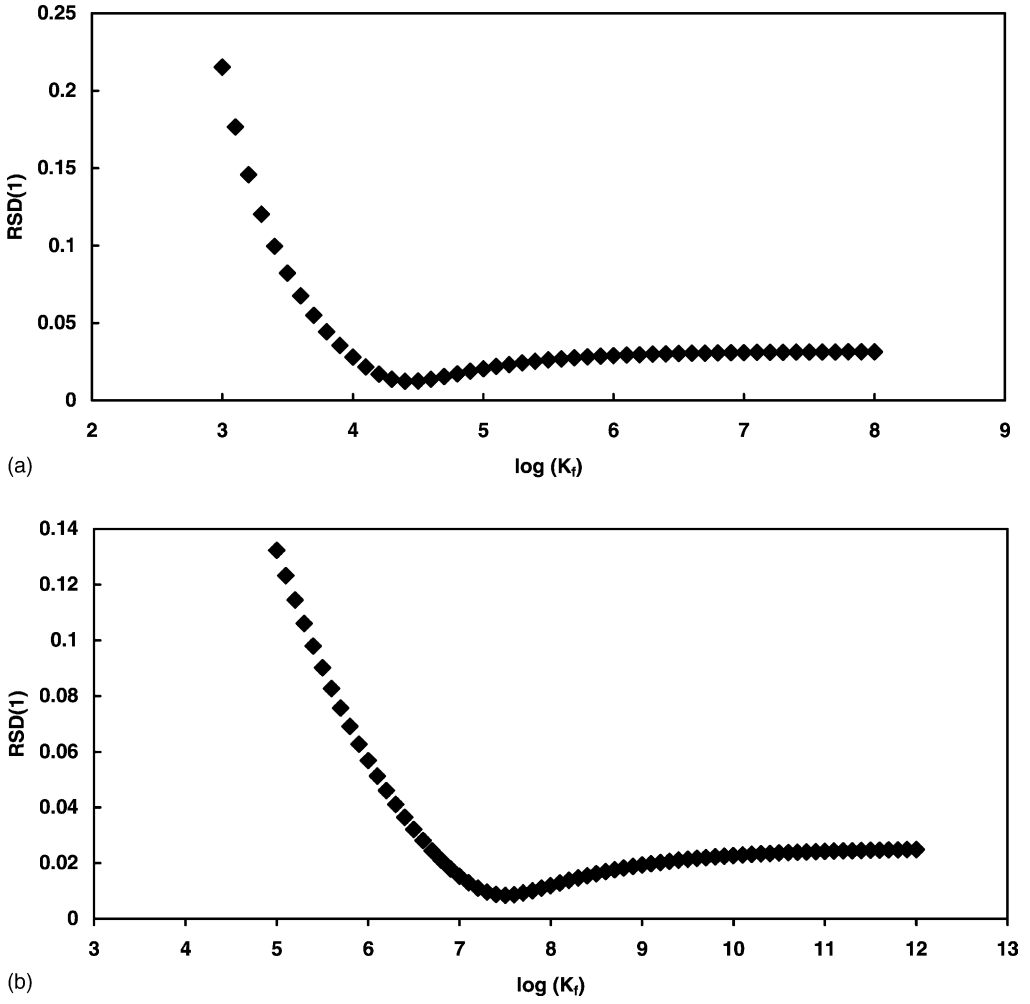

Fig. 8. The relationship between R.S.D.(1) and conditional stability constant, K_f: (a) 1:1 complexation system, obtained $\log(K_f) = 4.53$; (b) 1:2 complexation system, obtained $\log(K_f) = 7.50$.

components in considered complexation equilibria system. The stability constants K_1 and K_2 are optimized by the proposed method and the relationship between R.S.D.(2) and K values is shown as a surface in Fig. 6. The optimal set solution is $\log(K_1) = 6.25 \pm 0.01$ and $\log(K_2) = 4.13 \pm 0.02$. The standard deviation of the calculated $\log(K)$ values were estimated by the Jackknife procedure [50].

After calculating the concentration profiles of each component according to resolved stability constants (Fig. 7a), the absorption spectra can be calculated based on the concentration profiles and Eq. (10) as shown in Fig. 7b. It should be noted that the marked data points in spectrum of MTB–Cu, 1:1 complex were measured at excess amount of Cu^{2+} ion, which were in good accordance with spectrum obtained by using RAFA and further proved the reliability of the method.

3.3.2. One-step complex formation

The absorption spectra of two hypothetical 1:1 and 1:2 metal–ligand complex formation systems were created at the fixed concentration of ligand and various concentrations of metal ions (1:1 metal–ligand

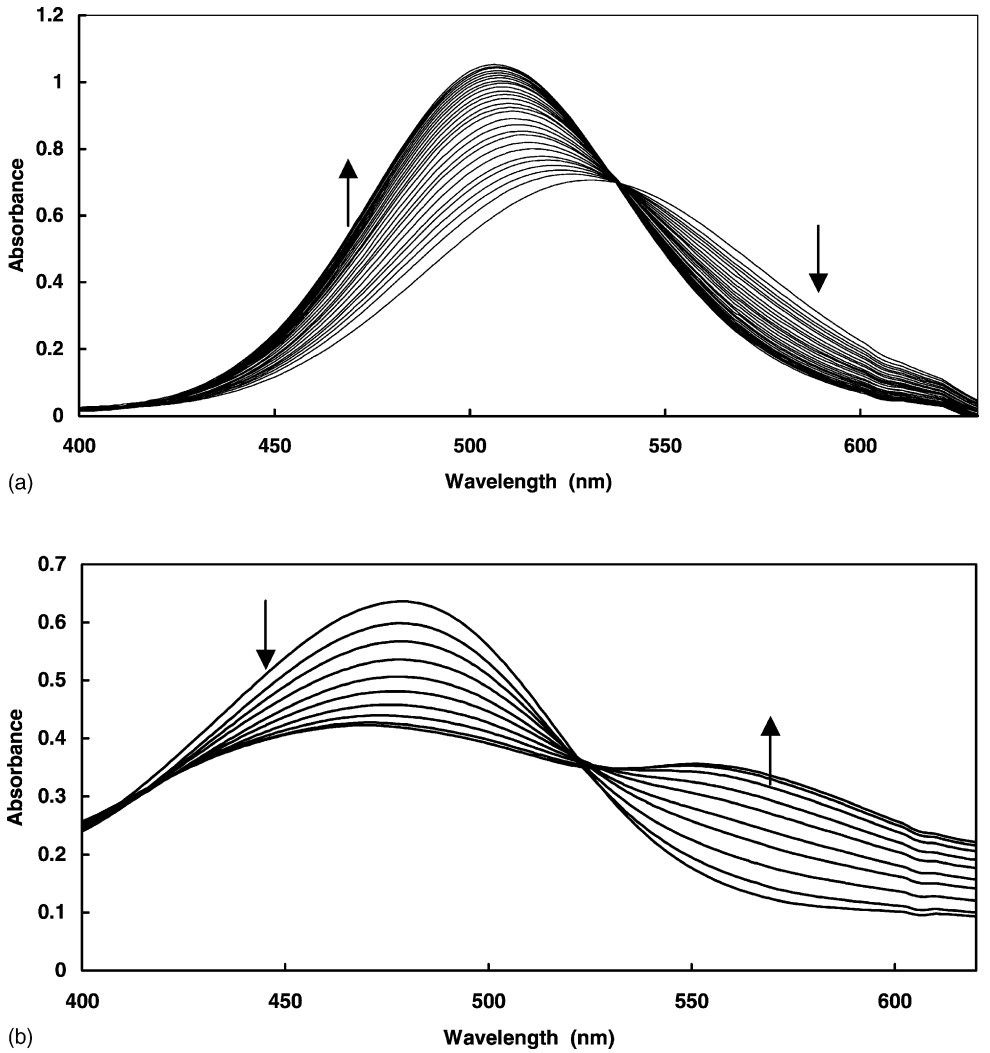

Fig. 9. Experimental absorption spectra of: (a) murexide–Ca system at 8.0×10^{-5} M murexide and various concentration of Ca^{2+} at pH 12; (b) dithiazone–Ni system at 4.7×10^{-5} M dithiazone and various concentration of Ni^{2+} at pH 7.

complex with $\log(K_f) = 4.50$, $\lambda_{max}(L) = 450$ nm, $\lambda_{max}(ML) = 465$ nm. and 1:2 metal–ligand complex with $\log(K_f) = 7.50$, $\lambda_{max}(L) = 450$ nm, $\lambda_{max}(ML_2) = 465$ nm). Table 1 presents the rank characteristics of created data matrices. In these cases also the eigenvalues, ratios of consecutive eigenvalues and R.S.D. suitably indicate the number of absorptive components in considered systems. The synthetic data matrices are processed by RAFA method and relationships between R.S.D.(1) of F and the conditional stability constant K_f are shown in Fig. 8. The curves in Fig. 8 indicate the optimum calculated values for K_f, which are in good accordance with given values. The constructed models showed that in the wide range of reliable formation constants, the predicted K_f values, absorption spectra and concentration profiles are in good accordance with given ones. If the complexation stoichiometry (n) is unknown, it is also possible to simultaneously estimate n and K_f by combination the optimization processes.

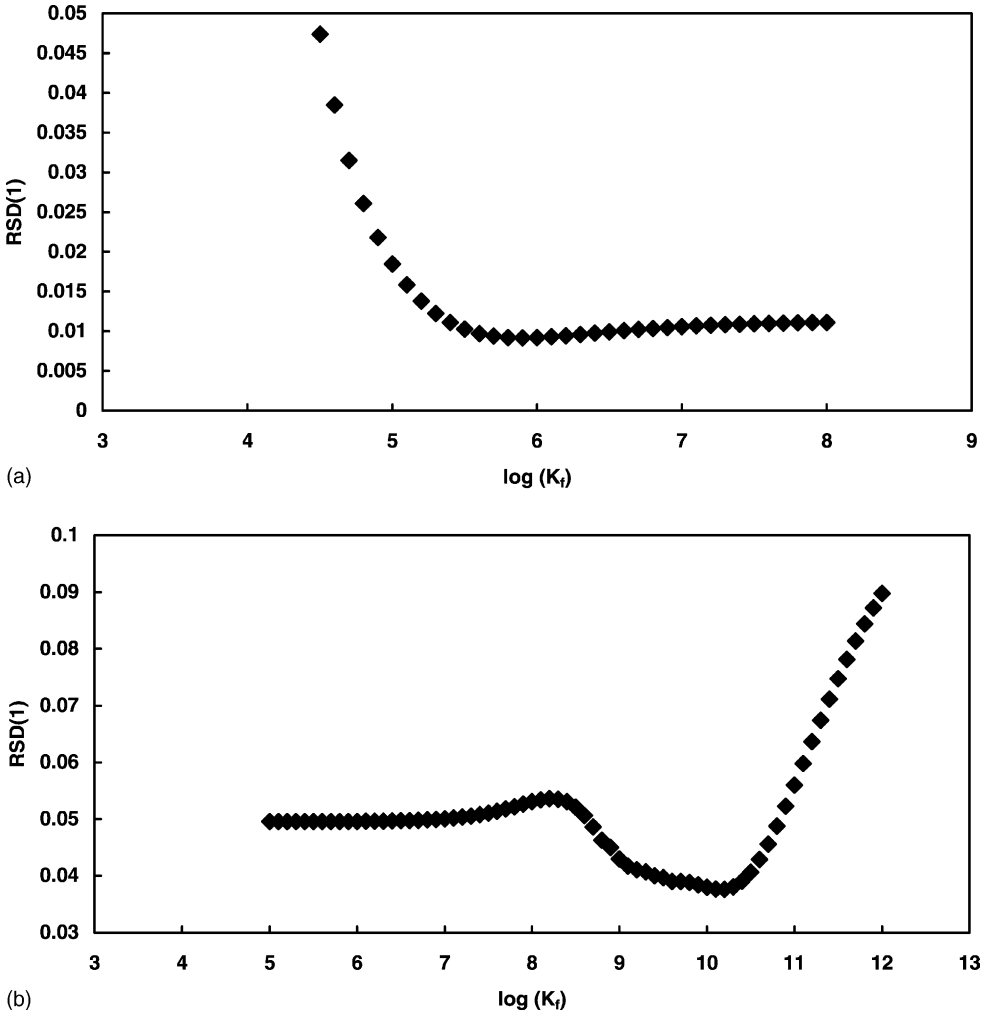

Fig. 10. The relationship between R.S.D.(1) and conditional stability constant, K_f: (a) 1:1 murexide–Ca system; (b) 1:2 dithiazone–Ni system.

The proposed spectrophotometric RAFA method was confirmed by complexation study of two different chemical systems. The one-step complex formation in murexide–calcium [51] and dithiazone–nickel [51] systems were selected as a model for 1:1 and 2:1 complexation equilibria with strong spectral overlapping between two components of chemical equilibria. Mole ratio method was used and the spectra of solutions containing a constant amount of the ligand at the fixed pH and varying amounts of metal ions were obtained. Fig. 9a and b show the typically evolutionary process of complexation for murexide–Ca (pH 12) and dithiazone–Ni systems (pH 7), respectively. A fixed and clear isosbestic point in each figure indicate the occurrence of one-step complex formation during the titration of ligands with metal ions.

The results due to applying the PCA on data matrices shown in Fig. 9 are presented in Table 3. The ratio of consecutive eigenvalues reaches maximum at $i = 2$, therefore indicating there exist only two absorptive components in considered complexation equilibria systems. The stability constant K_f is optimized by the proposed method and the relationship

between R.S.D.(1) and K_f values is shown in Fig. 10 for both complexation systems. The optimal solutions for murexide–Ca and dithiazone–Ni are $\log(K_f) = 6.25 \pm 0.01$ and $\log(K_f) = 4.13 \pm 0.01$, respectively. It is observed that the predicted and known absorption spectra were in good accordance.

4. Conclusions

RAFA is proposed as an efficient chemometric algorithm for completely analysis of equilibrium systems by spectrophotometric mole ratio method. The RAFA is based on the principle that the rank of two-way bilinear matrix of pure compound is one. The proposed method makes it possible to obtain the stability equilibrium constants, pure absorption spectra and species concentration profiles in several ligand–metal ion complex formation systems by sever spectral overlapping. The method was tested with simulated data sets and reliability was obtained by reproducing the input formation constants and species concentration profiles. The method was also applied to experimental data in 1:1 and 1:2, successive 1:1 and 1:2 metal–ligand complex formation. The proposed method can be applied for resolving the real two-step successive complex formation systems with full spectral overlapping of two complex species (rank-deficiency problem). However, the proposed method can also be applied for the one-step complex formation systems with unknown stoichiometry, it is possible to simultaneously estimate the n and K_f values.

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