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Investigation of the Effect of Inclusion of Eriochrome Black T with β-Cyclodextrin on Its Complexation Reaction with Ca^{2+} and Mg^{2+} Using Rank Annihilation Factor Analysis

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Rank annihilation factor analysis (RAFA) was used to investigate the effects of inclusion of eriochrome black T (EBT) on its complexation reaction with Ca^{2+} and Mg^{2+} at different pH values. Addition of β-cyclodextrin (β-CD) to Ca-EBT or Mg-EBT caused decomposition of the 1:1 metal complex and increase in EBT concentration in solution due to the formation of EBT-β-CD inclusion complex. In order to perform RAFA, concentration profiles were calculated by optimizing the value of the conditional stability constant and inclusion constant. The rank of original data matrix reduces by one by removing the information from the complex form (Ca-EBT or Mg-EBT). The performance of the method was evaluated by using synthetic data as well as experimental data and good results were obtained. The proposed method is capable of the simultaneous spectrophotometric determination of formation constant of a complex and the equilibrium constant of the competing reaction in solution.

Keywords: Eriochrome black T; Inclusion effect; Complexation; β-Cyclodextrin; RAFA

INTRODUCTION

Classical techniques for detecting complexes in solution are based on chemical models. Such information is imperative for interpreting sigmoidal pH curves, polarographic half-wave potential shifts and spectroscopic absorbances. 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 in different fields such as in the analysis of drugs and in the interpretation of their mechanism of action [1]. Up to the present, a large number of papers have been devoted to the study of complexation reactions 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 methods should be applied to obtain reliable data on the complexation reactions. Determination of the formation constants of complex for a system comprising a single equilibrium is easily performed through the knowledge of the chemical composition of the solution and the concentrations of the various species present. In contrast, a challenging situation arises when simultaneous equilibria must be considered.

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 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

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from using some complex mathematical algorithms, to solve such problems [6–8]. However, much more information can be extracted if multivariate spectroscopic data are analyzed by means of an appropriate multivariate data analysis method. 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 spectrophotometric data. Several soft and hard-modeling algorithms have been developed that analyze bilinear data obtained from chemical systems [9–17].

Rank annihilation factor analysis (RAFA) [18], is an efficient chemometric technique based on rank analysis for two-way spectral data and can be employed to analyze the gray system with unknown background quantitatively [19–27]. Recently we reported the application of RAFA in the determination of the conditional acidity constant as a function of β-CD concentration for some organic acids [28]. The stability of metal complexes can be affected by the presence of species that react with one or more of the metal, ligand or complex. Change in the stability of complexes can cause changes in their performance, applicability or efficiency. Therefore the study of the effect of different factors on the stability of complexes is important in different fields.

Cyclodextrins (CDs) are cyclic oligosaccharides that can form inclusion complexes with a variety of organic, inorganic and biological compounds in aqueous solution. Therefore the presence of CDs may affect the stability of complexes by formation of inclusion complexes with ligand, complex or both of them. The complexation phenomena in CDs often involve remarkable variations in photophysical and photochemical properties of guest molecules because of the environmental difference between the CD interior and aqueous medium. Most sensible spectral changes were reported for chemical indicators [28–30].

The dye Eriochrome black T (sodium 1-(1-hydroxy-2-naphthylazo)-6-nitro-2-naphtol-4-sulphonate, EBT), is a well known colorimetric reagent and has been used to a large extent as a metal ion indicator in analytical chemistry [31]. The proton associated with the sulfonic acid group in this compound is completely dissociated in an aqueous medium. The phenolic protons, on the other hand, are only partially dissociated with pK_1 and pK_2 values of 6.3 and 11.5.

In this work, we investigated the effect of inclusion of EBT on a complexation reaction of the Ca^{2+} and Mg^{2+} ions with EBT at different pH values. The effect of various concentrations of β-CD on the complexation reaction was monitored and RAFA was used to the simultaneous determination of the conditional stability constants and inclusion constants. The first aim of this work was introduction of a simple method for simultaneous determination of the stability constant of the complex and the equilibrium constant of the reaction that competes with the complexation reaction. The second aim is investigation of the effect of β-CD on the complexation reaction of Ca^{2+} and $Mg^{2+}$with EBT.

THEORETICAL BACKGROUND

EBT essentially exists in neutral water as a monovalent negative ion (H_2In^-) having the pK_a values: $pK_{a2} = 6.3$ and $pK_{a3} = 11.5$. The acid-base equilibrium predominating at pHs 8.4 to 9.5, and the corresponding acid-base ionization constant K_{a3} are therefore given for the dilute solutions used according to:

$$\mathrm{HIn}^{2-} \rightleftharpoons \mathrm{In}^{3-} + \mathrm{H}^{+} \qquad K_{a3} = \frac{[H^{+}][In^{3-}]}{[HIn^{2-}]} \tag{1}$$

By assuming the formation of a 1:1 complex between the cation M^{2+} and *EBT*, the complexation equilibrium can be written as:

$$\mathrm{M}^{2+} + \mathrm{In}^{3-} \rightleftharpoons \mathrm{MIn}^{-} \qquad K_f = \frac{[MIn^{-}]}{[M^{2+}][In^{3-}]} \tag{2}$$

where M^{2+} stands for the metal ions, In^{3-} and HIn^{2-} for different colored species of EBT and MIn^{-} for the 1:1 complex between M^{2+} and In^{3-}, while $[M^{2+}]$, $[In^{3-}]$, $[HIn^{2-}]$ and $[MIn^{-}]$ stand for the equilibrium concentrations of M^{2+}, In^{3-}, HIn^{2-}, and MIn^{-}, respectively.

By summation of the Eqs. (1) and (2), the overall complexation reaction and the equilibrium constant for overall reaction ($K_{f,HIn}$) are obtained.

$$\begin{gathered} \mathrm{M}^{2+} + \mathrm{HIn}^{2} \rightleftharpoons \mathrm{MIn}^{-} + \mathrm{H}^{+} \\ K_{f,HIn} = K_{a,3}.K_f = \frac{[MIn^{-}][H^{+}]}{[M^{2+}][HIn^{2-}]} \end{gathered} \tag{3}$$

Then the conditional formation constant K_f' that is pH dependent can be defined as:

$$K_f' = \frac{K_{f,HIn}}{[H^{+}]} = \frac{[MIn^{-}]}{[M^{2+}][HIn^{2-}]} \tag{4}$$

In the presence of β-CD, inclusion of HIn^{2-} takes place:

$$\mathrm{CD} + \mathrm{HIn}^{2-} \rightleftharpoons \mathrm{CD(HIn}^{2-}) \qquad K_{in} = \frac{[CD(HIn^{2-})]}{[CD][HIn^{2-}]} \tag{5}$$

where *CD* and $CD(HIn^{2-})$ stand for β-CD and the inclusion complex between HIn^{2-} and *CD*, while K_{in}, $[CD]$ and $[CD(HIn^{2-})]$ are the inclusion constant, the equilibrium concentrations of CD and the inclusion complex $CD(HIn^{2-})$, respectively.

If both M^{2+} and CD are present in the solution, then HIn^{2-} reacts with both of them to form MIn^{-} and $CD(HIn^{-})$ according to the Eqs. (3) and (5). Therefore, in the presence of CD and M^{2+} the mass balance equation for EBT is:

$$C_{HIn^{2-}} = [HIn^{2-}] + [MIn^{-}] + [CD(HIn^{2-})] \qquad (6)$$

where $[HIn^{2-}]$ is the equilibrium concentration of uncomplexed HIn^{2-}, neither with CD nor with M^{2+}. The total concentration of HIn^{2-} that does not form complex with M^{2+}, $[HIn_t^{2-}]$, is:

$$[HIn^{2-}] + [CD(HIn^{2-})] = [HIn_t^{2-}] \qquad (7)$$

By rearrangement of Eq. (5), the equilibrium concentration of $CD(HIn^{2-})$ can be obtained and by its substitution into Eq. (7) it can be written:

$$[HIn^{2-}] = \frac{[HIn_t^{2-}]}{(1 + K_{in}[CD])} \qquad (8)$$

Substitution of Eq. (8) into Eq. (4) and rearrangement yields:

$$K''_f = \frac{K'_f}{(1 + K_{in}[CD])} = \frac{[MIn^{-}]}{[M^{2+}][HIn_t^{2-}]} \qquad (9)$$

K''_f is a new conditional formation constant that applies at a single pH as well as a single concentration of CD. It should be noted that when the concentration of CD is zero (in the absence of CD), $K'_f = K''_f$ and $[HIn^{2-}] = [HIn_t^{2-}]$ and Eqs. (9) and (4) are the same.

We obtained K'_f by using RAFA on the profiles for the solutions at zero concentration of β-CD. The concentration profile of MIn^{-} and HIn^{2-} can be calculated for certain K'_f value, by obtaining the roots of Eq. (9) as a function of metal ion concentration. The equilibrium concentrations of MIn^{-} and HIn^{2-} at different metal ion concentrations can form two column vectors $[MIn^{-}]$ and $[HIn^{2-}]$, and they are referred to as the concentration profiles of MIn^{-} and HIn^{2-} species. A two-way data matrix with rank 2 can be formed by measuring absorbance under different wavelengths at a series of chosen metal ion concentrations with constant analytical concentrations of other components.

$$\begin{aligned} A &= A_{MIn^{-}} + A_{HIn^{2-}} + R \\ &= \varepsilon_{MIn^{-}}[MIn^{-}]^T + \varepsilon_{HIn^{2-}}[HIn^{2-}] + R \\ &= EC^T + R \end{aligned} \qquad (10)$$

where $A_{MIn^{-}}$ and $A_{HIn^{2-}}$ are the bilinear measuring matrix of pure 1:1 metal complex and ligand, respectively, and each one can be decomposed into the corresponding molar absorptivity spectrum $\varepsilon_{MIn^{-}}$ and $\varepsilon_{HIn^{2-}}$ (column vectors), and the concentration profiles $[MIn^{-}]^T$ and $[HIn^{2-}]^T$ (row vectors, superscript T denotes the transpose of a matrix or vector). E and C^T represent matrices formed by the molar absorptivity spectrum and the concentration profile of each species, respectively. R is the residual matrix and should contain only noise. The size of matrix A is $w \times c_M$ where w denotes the number of wavelengths and c_M is the number of metal ion concentrations for which absorbances were recorded (c_M is smaller than w).

Obviously, the size of matrices E and C^T are $w \times 2$ and $2 \times c_M$, respectively. The pure spectra of $[MIn^{-}]$ can be readily measured at the excess amount of metal ion; the primary purpose of investigating the considered complexation model is to acquire the equilibrium parameters. Let

$$F = A - A_{MIn^{-}} = A - \varepsilon_{MIn^{-}}[MIn^{-}]^T \qquad (11)$$

RAFA was also used on the profile in the presence of β-CD concentration and we obtained K''_f for each CD concentration and the best estimation of K_{in} (Eq. (9)). The concentration profiles of MIn^{-} and HIn_t^{2-} can be calculated for certain K_{in} values, respectively, by obtaining the roots of Eq. (9) as a function of β-CD concentration. The equilibrium concentrations of MIn^{-} and HIn_t^{2-} at different β-CD concentrations can form two column vectors $[MIn^{-}]$ and $[HIn_t^{2-}]$. They are referred to as the concentration profiles of MIn^{-} and HIn_t^{2-} species. A two-way data matrix with rank 2 in the presence of β-CD can be formed by measuring absorbance at different wavelengths for a series of chosen β-CD concentrations with constant analytical concentrations of species. In the presence of β-CD, the total sum of data matrix B plus its submatrices can be given as:

$$\begin{aligned} B &= B_{MIn^{-}} + B_{HIn_t^{2-}} + R \\ &= \varepsilon_{MIn^{-}}[MIn^{-}]^T + \varepsilon_{HIn_t^{2-}}[HIn_t^{2-}]^T + R \\ &= EC^T + R \end{aligned} \qquad (12)$$

The size of matrix B is $w \times c_{CD}$, where w denotes the number of wavelengths and c_{CD} is the number of β-CD concentration for which absorbances were recorded (c_{CD} is smaller than w).

Obviously, the size of matrices E and C^T in the presence of β-CD are $w \times 2$ and $2 \times c_{CD}$, respectively. Let

$$G = B - B_{MIn^{-}} = B - \varepsilon_{MIn^{-}}[MIn^{-}]^T \qquad (13)$$

The aim of RAFA approach is to find a suitable set of K'_f in the absence of β-CD, and also K_{in} and K''_f, as a function of K_{in} and β-CD concentration. So that the rank of matrixes F and G can be reduced by 1 for those of matrices A and B respectively, through introduction of the concentration profiles of complex species.

EXPERIMENTAL

Synthetic Data

To evaluate the performance of the method, a set of synthetic absorption spectra of 1:1 complex formation system were created. The use of simulated data allows us to know how each problem affects the performance of the proposed method.

Parameters setting in the absence of β-CD is as follows: $K'_f = 5000\,\mathrm{L\,mol^{-1}}$ and $C_{HIn^{2-}} = 5.0 \times 10^{-5}\,\mathrm{mol\,L^{-1}}$, by measuring metal ion concentrations in the range 5.0×10^{-5}–$5.0 \times 10^{-4}\,\mathrm{mol\,L^{-1}}$ with $5.0 \times 10^{-5}\,\mathrm{mol\,L^{-1}}$ intervals (10 concentrations in all). The measuring wavelengths are 300–700 nm with 1 nm intervals (401 wavelengths in all). Parameter setting in the presence of β-CD is as follows: $K_{in} = 200\,\mathrm{L\,mol^{-1}}$, $C_{HIn^{2-}} = 5.0 \times 10^{-5}\,\mathrm{mol\,L^{-1}}$, $M^{2+} = 1.5 \times 10^{-4}\,\mathrm{mol\,L^{-1}}$. By measuring β-CD concentrations in the range 0.002–0.02 mol $\mathrm{L^{-1}}$ with 0.003 mol $\mathrm{L^{-1}}$ intervals (seven concentrations in all) the pure spectra of all components are produced by Gaussian function in the wavelength range of 300–700 nm. 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.

Real Data

Absorption spectra were obtained with a Perkin-Elmer Lambda 45 UV–vis spectrophotometer using 1 cm path length quartz cells and the measurements were performed at 25 ± 0.1°C and in the solutions with constant ionic strength of 0.15 mol $\mathrm{L^{-1}}$. pH measurements were made with a Metrohm 713 pH-meter using a combined glass electrode. EBT, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, and β-CD were purchased from Merck and used without further purification. All specific details are given in the next section.

Calculations

All calculations were performed in MATLAB 6.5 (Math Works, Cochituate Place, MA).

RESULTS AND DISCUSSION

Synthetic Data

Figure 1 shows created absorption spectra of 1:1 complex formation system, at the fixed concentration of ligand and various concentration of metal ion. Created absorption spectra of 1:1 complex formation system at fixed concentration of ligand and metal ion and in the presence of various concentrations of β-CD is also shown in Fig. 2. Based on principal component analysis (PCA), the R.S.D. method is widely used to determine the number of principal components [32,33]. 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

$$R.S.D.(n) = \left(\frac{\sum_{i=n+1}^{c} g_i}{(w-n)(c-n)}\right)^{1/2} \tag{14}$$

where g_i is the eigenvalue and n is the number of considered principal components. Table I presents the eigenvalues, ratios of consecutive eigenvalues and R.S.D. of matrices A and B created for simulation

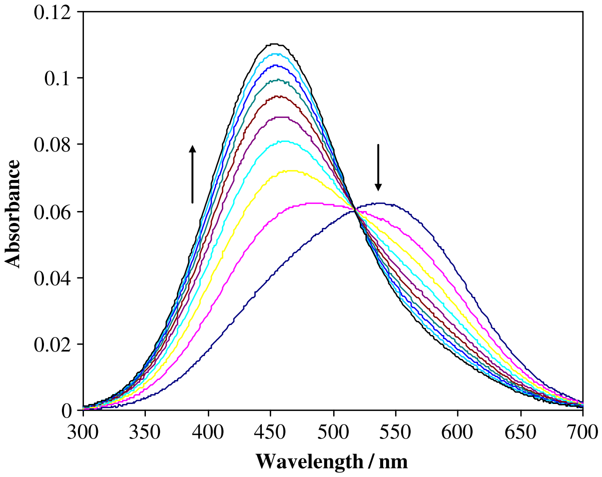

FIGURE 1 Simulated absorption spectra of a 1:1 metal–ligand complex formation systems at the fixed concentration of ligand ($C_{HIn^{2-}} = 5.0 \times 10^{-5}\,\mathrm{mol\,L^{-1}}$), various concentrations of metal ion (5.0×10^{-5}–$5.0 \times 10^{-4}\,\mathrm{mol\,L^{-1}}$ with $5.0 \times 10^{-5}\,\mathrm{mol\,L^{-1}}$ intervals) with $K'_f = 5000\,\mathrm{L\,mol^{-1}}$.

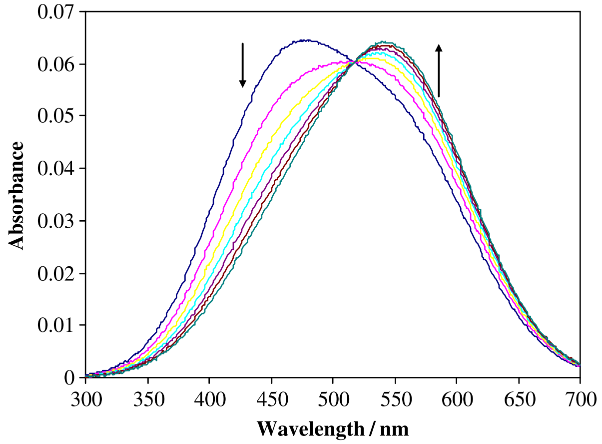

FIGURE 2 Simulated absorption spectra of 1:1 complex formation system at fixed concentration of ligand and metal ion ($C_{HIn^{2-}} = 5.0 \times 10^{-5}\,\mathrm{mol\,L^{-1}}$, $M^{2+} = 1.5 \times 10^{-4}\,\mathrm{mol\,L^{-1}}$), and in the presence of various concentrations of β-CD (0.002–0.02 mol $\mathrm{L^{-1}}$, with 0.003 mol $\mathrm{L^{-1}}$ intervals) with $K_{in} = 200\,\mathrm{L\,mol^{-1}}$.

TABLE I The results of PCA on simulated data

	In the absence of β-CD			In the presence of β-CD		
i	g_i	g_i/g_{i+1}	R.S.D	g_i	g_i/g_{i+1}	R.S.D
1	2.9652	5.8207	0.0121	1.9700	9.2910	0.0096
2	0.5094	227.9343	0.0021	0.2120	97.5870	0.0020
3	0.0022	1.0349	0.0020	0.0022	1.0200	0.0018
4	0.0022	1.0229	0.0018	0.0021	1.0831	0.0015
5	0.0021			0.0020		

data in the absence and presence of β-CD. It should be noted that for both matrices A and B the ratio of consecutive eigenvalues when n = 2 is the maximum, it shows that there exist two absorptive components in the system, which coincide with the assumption of the simulative experiment. It should also be noted that the absorption spectra for HIn^{2-} and $CD(HIn^{2-})$ are the same. Therefore, the appearance of the two absorptive species, ($HIn^{2-} + CD(HIn^{2-})$ and MIn^{-}) for the system in the presence of CD is not surprising.

The simulated data of matrices A and B are processed by RAFA method. The best estimation of K'_f and K_{in} reduces the rank of the systems by one. The relationship between R.S.D. of F and G matrices and the estimations of conditional formation constant K'_f and inclusion constant K_{in} are shown as R.S.D. curves in Figs. 3 and 4, respectively. Figures 3 and 4 indicate the optimum calculated values for K'_f and K_{in}, which are in good agreement with given values. For each value of K'_f and K_{in}, concentration profiles of MIn^- were calculated and the corresponding F and G matrices were obtained (Figs. 5 and 6). The absorption spectra belong to the HIn_t^{2-}. Narrow minima are observed in the R.S.D. curves shown in Figs. 3 and 4 which indicate the optimal estimates of K'_f and K_{in}. The values of K''_f at different concentrations of β-CD can simply be estimated from Eq. (9) using the optimal estimates of K'_f and K_{in}.

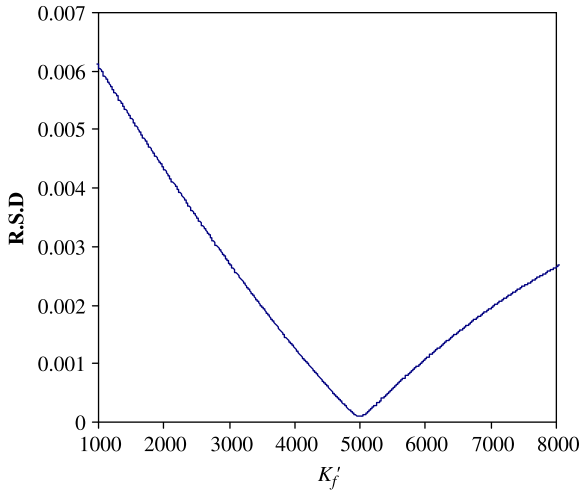

FIGURE 3 Relationship between RSD and K'_f estimations for simulated data.

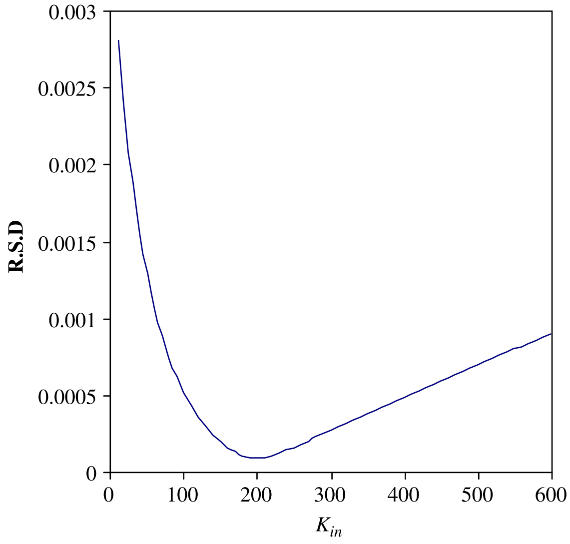

FIGURE 4 Relationship between RSD and K_{in} estimations for simulated data.

Experimental Data

The proposed spectrophotometric RAFA method was confirmed by a complexation study of two different chemical systems. The spectral data for the complex formation of Ca-EBT and Mg-EBT systems in the presence of different concentrations of β-CD were used as experimental data. The cations Ca^{2+} and Mg^{2+} form 1:1 mono-anionic (MIn^-) type complexes with EBT within the pH range 8.4–9.5. This is true since the best pH range (color change) for complexometric studies of metal ions with EBT was determined earlier to be 8.0–10.0 [31]. Moreover, EBT essentially exists as HIn^{2-} species within this pH range, where the mole fractions of H_2In^- and In^{3-} species are negligible, and hence complexation of either metal ion with In^{3-} would not be too strong to prohibit the formation of HIn^{2-}/β-CD inclusion complex.

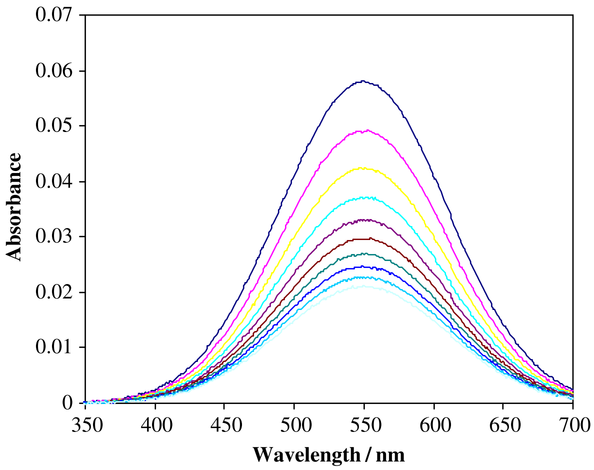

FIGURE 5 Simulated absorption spectra in Fig. 1 after subtraction of MIn^- spectra using RAFA.

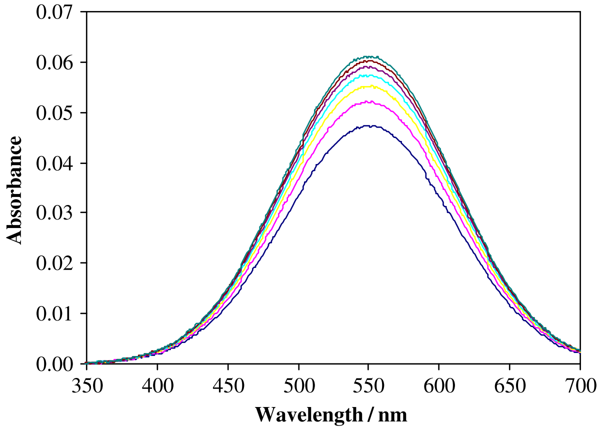

FIGURE 6 Simulated absorption spectra in Fig. 2 after subtraction of MIn^- spectra using RAFA.

First, inclusion complex formation between EBT and β-CD at different pH values were studied using RAFA by the method described in our previous work [28]. Based on the obtained results the estimated stability constants for the complexation reactions of HIn^{2-} and In^{3-} were 206 L mol^{-1} and 46 L mol^{-1}, respectively. This indicates that although both HIn^{2-} and In^{3-} form inclusion complexes with β-CD, the complex of HIn^{2-} is more stable than that of In^{3-}. On the other hand at the pH range used for the investigation of the complexation of EBT with Ca^{2+} and Mg^{2+}(8.4–9.5) in the presence of β-CD the mole fraction of In^{3-} is nearly zero. Therefore the reaction of In^{3-} with β-CD can be neglected.

Figure 7 shows the absorption spectra for 4×10^{-5} mol L^{-1} EBT at pH 9.5 (borax buffer with the ionic strength of 0.15 mol L^{-1}) and varying amounts of Ca^{2+}. EBT shows an absorption band with the absorption maximum at 620 nm. This absorption band is attributed to the HIn^{2-} form. With an increase in Ca^{2+} concentration, the absorption at 620 nm gradually decreased while the absorption at 560 nm for 1:1 Ca-EBT complex increased.

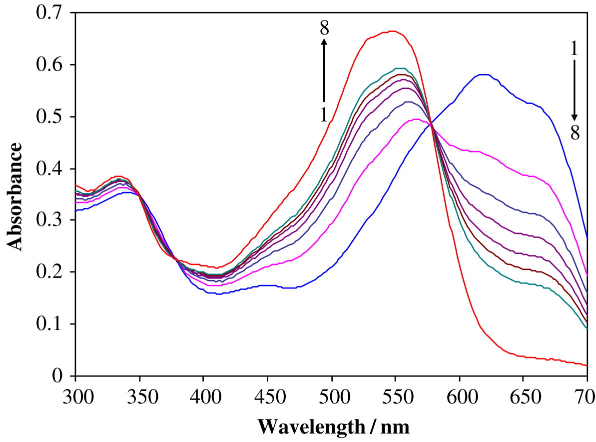

FIGURE 7 The absorption spectra for 4×10^{-5} mol L^{-1} EBT in the presence of (1) 0.0, (2) 2.0×10^{-4}, (3) 4.0×10^{-4}, (4) 6.0×10^{-4}, (5) 8.0×10^{-4}, (6) 1.0×10^{-3}, (7) 1.3×10^{-3} and (8) 6.5×10^{-3} mol L^{-1} Ca^{2+} at pH 9.5.

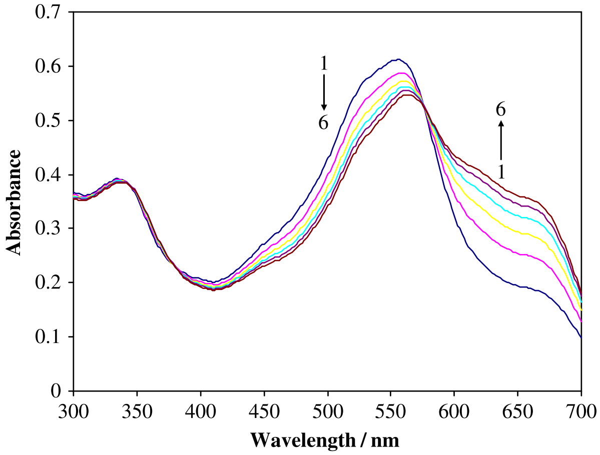

FIGURE 8 The spectra for Ca-EBT complex (1.0×10^{-3} mol L^{-1}Ca^{2+} and 4.0×10^{-5} mol L^{-1} EBT) in the presence of (1) 0.0, (2) 3.0×10^{-3}, (3) 6.0×10^{-3}, (4) 9.0×10^{-3}, (5) 1.2×10^{-2} and (6) 1.5×10^{-2} mol L^{-1} of β-CD at pH 9.5.

The spectra for Ca-EBT complex in the presence of varying amounts of β-CD at pH 9.5 are shown in Fig. 8. An increase in the β-CD concentration from 0.0 to 0.015 mol L^{-1} caused a decrease in the absorbance of the solution at 560 nm and an increase in the absorbance at 620 nm. This change in the absorbance of the solution at 620 and 560 nm is due to the decomposition of 1:1 metal complex and increase in the HIn^{2-} concentration in the solution due to formation of inclusion complex with β-CD. The results indicate that formation of inclusion complex of HIn^{2-} with β-CD weakens the Ca-EBT complex.

The number of absorptive species also may be determined by applying PCA on data matrices (in the absence and presence of β-CD) shown in Figs. 7 and 8. The results are presented in Table II. The ratio of consecutive eigenvalues reaches maximum at i = 2, which indicates there exist two absorptive species in each system (in the absence and presence of β-CD). The conditional formation constant K'_f, the inclusion constant K_{in} and the conditional formation constant K''_f, were obtained by the proposed method

TABLE II The results of PCA on experimental data for Ca-EBT system at two different conditions: (I) 4.0×10^{-5} mol L^{-1} EBT in the presence of different concentrations of Ca^{2+} at pH 9.5 and (II) 4.0×10^{-5} mol L^{-1} EBT, 1.0×10^{-3} mol L^{-1} Ca^{2+} and different concentrations of β-CD at pH 9.5

	Condition I			Condition II		
i	g_i	g_i/g_{i+1}	R.S.D	g_i	g_i/g_{i+1}	R.S.D
1	11.8284	8.6899	0.00374	12.3740	11.6241	0.0327
2	1.3612	57.1623	0.0064	1.0645	418.5584	0.0019
3	0.0238	3.6054	0.0042	0.0025	6.0498	0.0011
4	0.0066	1.0788	0.0033	0.0004	1.0852	0.0008
5	0.0045			0.0004		

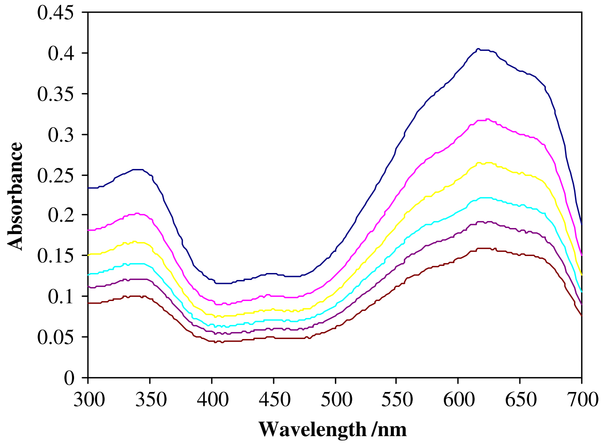

FIGURE 9 The absorption spectra in Fig. 7 after subtraction of Ca-EBT spectra at pH 9.5 using RAFA.

as described above. The optimal estimates were found as: $K'_f = 2050\,\mathrm{L\,mol^{-1}}$ with R.S.D $= 0.0012$ and $K_{in} = 250\,\mathrm{L\,mol^{-1}}$ with R.S.D $= 0.0013$. Based on the optimal K'_f and K_{in} estimates, the rank of original data matrices when the complex profiles was reduced by one as the complex profiles were subtracted (Figs. 9 and 10).

The above experiments were also repeated at different pH values. The values of K_{in} at pH 8.9 and 9.2 were estimated as $210\,\mathrm{L\,mol^{-1}}$ and $190\,\mathrm{L\,mol^{-1}}$ with R.S.D.s of 0.0011 and 0.0019, respectively. The results (Table III) show that, as expected, the conditional formation constant K''_f for the complex at each concentration of β-CD increased by increasing pH values. At a given pH, the values of K''_f decreased by increasing β-CD concentration based on Eq. (9). This decrease is due to the formation of an inclusion complex between HIn^{2-} and β-CD. The amount of decrease in K''_f with increasing β-CD concentration depends on the stability of the inclusion complex. More stable inclusion complex results in a corresponding decrease in K''_f by increasing β-CD concentration.

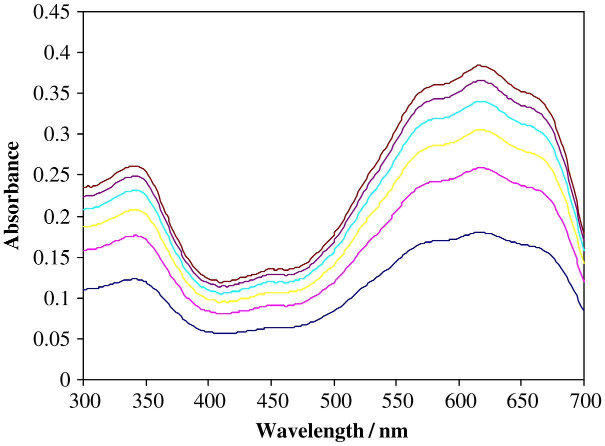

FIGURE 10 The absorption spectra in Fig. 8 after subtraction of Ca-EBT spectra at pH 9.5 using RAFA.

TABLE III Conditional formation constants for 1:1 complex of Ca-EBT in the presence of different concentrations of β-CD at different pH values

β-CD Concentration ($\mathrm{mol\,L^{-1}}$)	K''_f / $\mathrm{L\,mol^{-1}}$		
	pH = 8.9	pH = 9.2	pH = 9.5
0.000	770	1200	2050
0.003	472	764	1171
0.006	341	561	820
0.009	266	443	631
0.012	219	366	513
0.015	186	312	432

The spectral behavior of a constant concentration of EBT at pH 8.9 (borax buffer with an ionic strength of $0.15\,\mathrm{mol\,L^{-1}}$) and different concentrations of Mg^{2+} and hence Mg-EBT complex in the presence of varying amounts of β-CD at different pH values were studied as described above.

The experimental data matrices for the complexation reaction were investigated with PCA and, as for calcium, both the eigenvalues and the ratios of consecutive eigenvalues showed two absorptive components in the spectra of EBT, both in the presence and absence of β-CD systems (Table IV).

The RAFA method was used for analysis of the mentioned system where K_{in} and the conditional formation constant K'_f were estimated. The estimated values are $K'_f = 60670\,\mathrm{L\,mol^{-1}}$ with R.S.D $= 0.0016$ and $K_{in} = 230\,\mathrm{L\,mol^{-1}}$ with R.S.D $= 0.0003$. Based on the optimum K'_f and K_{in} estimates, the rank of the original data matrices was reduced by one as the complex profiles were subtracted. The above experiments were repeated at different pH values and the results are given in Table V. The values of K_{in} at pH 8.4, 9.2 and 9.5 were estimated as 230, 260 and $240\,\mathrm{L\,mol^{-1}}$ with R.S.D of 0.0003, 0.0004 and 0.0002, respectively. As for Ca-EBT system, increasing β-CD concentration caused a decrease in conditional formation constant K''_f.

The values of $\log K'_f$ for Ca-EBT and Mg-EBT complexes obtained by the RAFA method ($5.52\,\mathrm{L\,mol^{-1}}$ and $7.35\,\mathrm{L\,mol^{-1}}$, respectively) are in good agreement with the previously reported values ($5.4\,\mathrm{L\,mol^{-1}}$ and $7.0\,\mathrm{L\,mol^{-1}}$, respectively) [34].

TABLE IV The results of PCA on experimental data for Mg-EBT system at two different conditions: (I) $4.0 \times 10^{-5}\,\mathrm{mol\,L^{-1}}$ EBT in the presence of different concentrations of Mg^{2+} at pH 8.9 and (II) $4.0 \times 10^{-5}\,\mathrm{mol\,L^{-1}}$ EBT, $4.0 \times 10^{-5}\,\mathrm{mol\,L^{-1}}$ Mg^{2+} and different concentrations of β-CD at pH 8.9

	Condition I			Condition II		
i	g_i	g_i/g_{i+1}	R.S.D	g_i	g_i/g_{i+1}	R.S.D
1	12.4804	6.6000	0.0436	4.6559	79.7666	0.0078
2	1.8956	44.0260	0.0013	0.0584	89.2491	0.0014
3	0.0004	1.1000	0.0011	0.0007	1.5837	0.0011
4	0.0004	1.0000	0.0008	0.0004	1.0184	0.0008
5	0.0004			0.0004		

TABLE V Conditional formation constants for 1:1 complexes of Mg-EBT in the presence of different concentrations of β-CD at different pH values

β-CD Concentration ($mol\,L^{-1}$)	K_f'' / $L\,mol^{-1}$			
	pH = 8.4	pH = 8.9	pH = 9.2	pH = 9.5
0.000	19640	60670	73280	131740
0.003	11621	35899	41169	76593
0.006	8252	25492	28625	53992
0.009	6397	19762	21940	41690
0.012	5223	16136	17786	33954
0.015	4414	13634	14955	28639

CONCLUSION

RAFA was used to introduce a novel methodology for the evaluation of the effects of competing equilibria on the magnitudes of estimated complex formation constants. Simultaneous determination of the formation constant of the 1:1 complex and the equilibrium constant of the competing reaction in solution was performed. RAFA was proposed as an efficient chemometrics algorithm for complete analysis of competing equilibrium systems by the spectrophotometric method. The method was tested with simulated data sets and the reliability was assessed by reproducing the input formation constants and species concentration profiles. The method was successfully applied to experimental data showing the effects of HIn^{2-} inclusion into cyclodextrin on EBT complexation reaction with Ca^{2+} and Mg^{2+}. The method can be applied to any complexation or association systems with strong or weak interactions.

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