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Acidity Constants of Some Organic Acids in the Presence of *β*-Cyclodextrin in Binary Ethanol−Water Mixtures by Rank Annihilation Factor Analysis

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The acid dissociation constants of methyl red, 4-nitrophenol, 4-nitrocatechol, and neutral red were studied in water and ethanol−water cosolvents and in the presence of β-cyclodextrin (β-CD). Apparent acidity constants of these acid−base indicators were derived using rank annihilation factor analysis on spectrophotometric data. Formation constants of complexes with β-CD for both acidic and basic forms were obtained by curve fitting. The effects of the solvent and inclusion complex on the acidity constant and the effects of the solvent on the inclusion of the investigated acids with β-CD are discussed.

Introduction

The effect of solvent composition on the dissociation equilibria of organic reagents is of great importance in chemical and biomedical analysis.[1] Recently, mixed solvents have been widely used in analytical chemistry with the extension of research fields and the development of nonaqueous analytical techniques. These solvents are advantageous in the titrations of the acids and bases which are too weak or insoluble in water.[2] Dissociation constants can be a key parameter for understanding and quantifying chemical phenomena reaction rates, biological activity, biological uptake, biological transport, and environmental fate.[3] Several methods for the determination of dissociation constants, such as potentiometric titration, spectrophotometric determination, and conductometry, have been reported.[4,5]

Cyclodextrins (CDs) are known to complex with a wide variety of molecules in their hydrophobic interior. Due to their unique ability to form inclusion complexes with numerous compounds CDs are frequently applied in various fields of chemistry.[6] As a result of this inclusion, a change of the chemical or physical properties of the guest molecule is generally observed opening a wide field of applications in areas such as organic and analytical chemistry.[7] The interaction of solutes with β-cyclodextrin (β-CD) leads to apparent changes in their chemical properties, such as shifts in their dissociation equilibria. Formation of inclusion complexes between acid and/or base forms of a compound with β-CD can change the conditional acidity constant, $K_{a,c}$. The change in acidity constant depends on the relative stability of the inclusion complexes of acidic and basic forms of the compound with β-CD.[8]

Using chemometrics methods, one can analyze whole spectra, thereby utilizing all spectral information. The approach is superior to any single-point measurement since several hundreds of data points per spectrum can be treated simultaneously. Rank annihilation factor analysis (RAFA) is an efficient chemometric technique based on rank analysis for two-way spectral data and can be employed to analyze the gray system with an unknown background quantitatively.[9] Recently, we reported the application of RAFA in the spectrophotometric study of acidity constants,[8] complex formation equilibria,[10] and chemical kinetics[11,12] as a function of β-CD concentration.

In the present paper, RAFA is applied to investigate the changes of the equilibrium constants of neutral red, methyl red, 4-nitrophenol, and 4-nitrocatechol in the presence of β-CD in aqueous and aqueous−organic solvents. Their dissociation constants and the color transition range in water are well-known.[13] The observed conditional acidity constants (β-CD concentration dependent acidity constant) of these acid−base indicators and their association constants with β-CD are discussed as a function of the content of organic solvent which are derived by RAFA.

Experimental

Apparatus. 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 $0.15\ \text{mol}\cdot\text{L}^{-1}$. pH measurements were made with a Metrohm 713 pH-meter using a combined glass electrode. The recorded pH values for mixed solvents were corrected using a tabulated correction factor, δ, reported for an ethanol−water solution.[14]

Reagents. Neutral red, 4-nitrophenol, 4-nitrocatechol, methyl red, and β-cyclodextrin were reagent-grade materials, and H_3PO_4, NaH_2PO_4, Na_2HPO_4, $NaCH_3COO$, CH_3COOH, NH_4Cl, and NH_3 were of pro-analysis grade from E. Merck. These chemicals were used without further purification.

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

Results and Discussion

The basis of the application of RAFA in the spectrophotometric determination of acidity constants was described in our previous work.[8] A two-way data matrix with rank 2 can be formed by measuring absorbance spectra for a constant concentration of the desired compound at several chosen pHs.[15] The data matrices are obtained at various concentrations of β-CD and solvent compositions. The concentration profiles are calculated by optimizing the value of the conditional acidity constants, and the rank of the original data matrix reduces by one by removing the information of one of the acidic or basic

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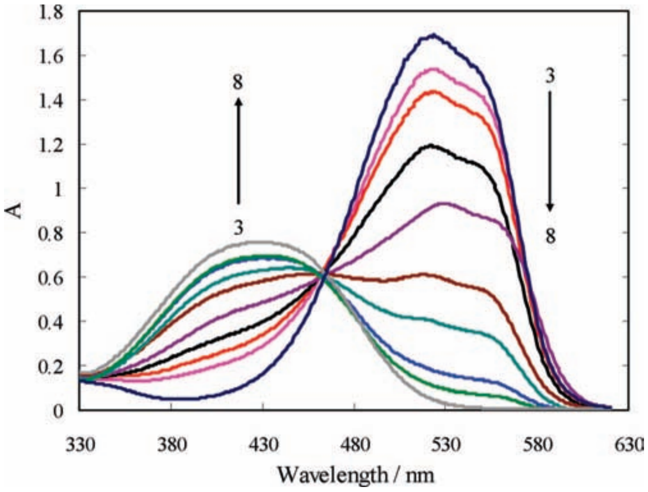

Figure 1. Absorption spectra for $4.0 \cdot 10^{-5}$ mol·L^{-1} methyl red in water at various pH values. The pH values are: 1, 3.0; 2, 4.2; 3, 4.5; 4, 4.7; 5, 5.0; 6, 5.2; 7, 5.4; 8, 5.7; 9, 5.9; and 10, 8.0.

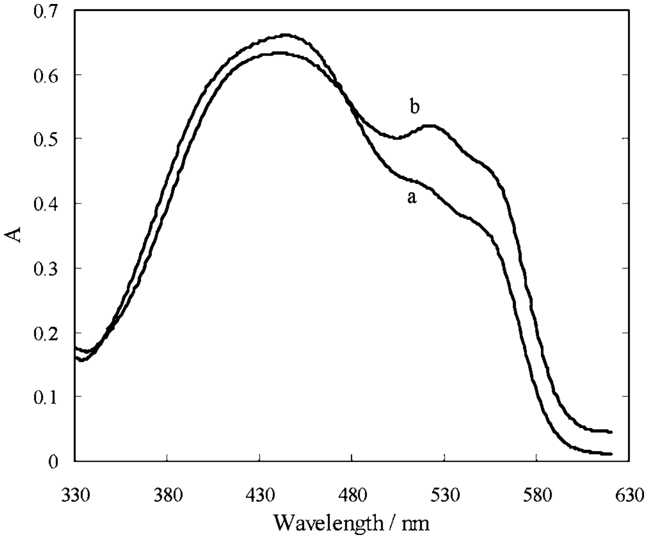

Figure 2. Absorption spectra for $4 \cdot 10^{-5}$ mol·L^{-1} methyl red in (a) water and (b) water−ethanol (80/20) at pH 5.4.

forms in the matrix. The formation constants of the acidic and basic forms with β-CD can be obtained by curve fitting.

Figure 1 shows the absorption spectra of $4.0 \cdot 10^{-5}$ mol·L^{-1} methyl red in aqueous solution at different pH values. The absorption spectrum of methyl red at pH 3.0 shows an absorption band which has an absorption maximum at 520 nm. This absorption band is attributed to the acidic form of methyl red that is a zwitterion, H^+MR^-.[16] With the increase of pH, the absorbance at 520 nm for the acidic form gradually decreases, whereas the absorption at 430 nm for the negative form of methyl red, MR^-, increases. A sharp isosbestic point is observed in Figure 1 that indicates the presence of equilibrium between the H^+MR^- and MR^- forms.

Figure 2 shows the spectra of $4.0 \cdot 10^{-5}$ mol·L^{-1} methyl red in aqueous solution at pH 5.4 (Figure 2a) and mixed solvent containing 20 % (v/v) ethanol at the same pH (Figure 2b). As shown in this figure, in the presence of ethanol, parallel to an increase in the absorbance of zwitterion acidic form, $\lambda_{max} =$ 520 nm, the absorbance of its basic form with a negative charge at 430 nm decreases.

The spectra of methyl red in pure water in the presence of varying amounts of β-CD at pH 5.2 in water solvent are shown in Figure 3. An increase in the β-CD concentration from (0.0 to 4.8)$\cdot 10^{-3}$ mol·L^{-1} caused a decrease in the absorbance of the solution at 520 nm and an increase in the absorbance at 430 nm. These absorbance changes indicate that the basic form of methyl red has more affinity than the acidic form to formation of an inclusion complex with β-CD. The hydrophobicity of the acidic form of methyl red (zwitterion form) is more than that of its basic form (with one negative charge). From the above, it can be concluded that hydrophobic interactions play an important role in the stabilization of inclusion complexes of acidic and basic forms of methyl red with β-CD.

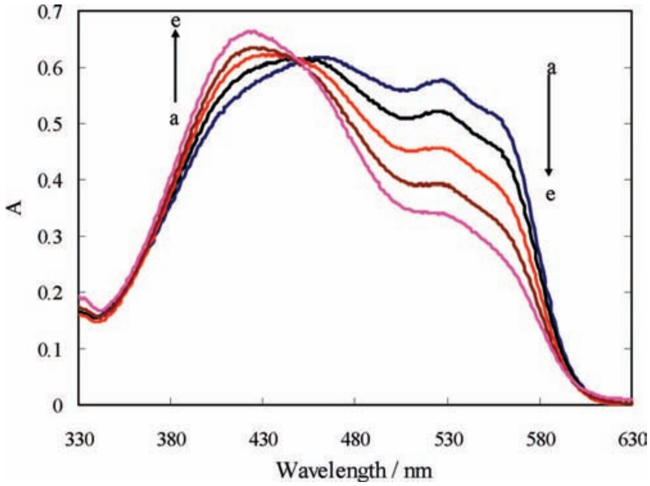

Figure 3. Absorption spectra for $4.0 \cdot 10^{-5}$ mol·L^{-1} methyl red in the presence of different concentrations of β-CD at pH 5.2. The concentrations of β-CD are: (a) 0.0, (b) $4.0 \cdot 10^{-4}$ mol·L^{-1}, (c) $1.0 \cdot 10^{-3}$ mol·L^{-1}, (d) $2.0 \cdot 10^{-3}$ mol·L^{-1}, and (e) $4.8 \cdot 10^{-3}$ mol·L^{-1}.

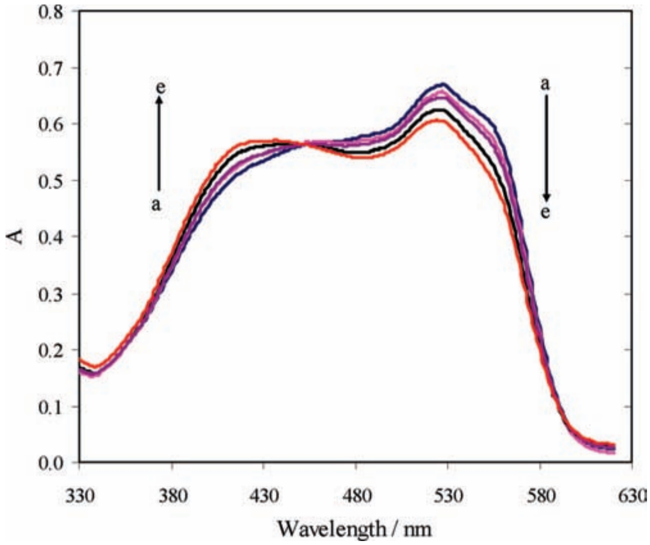

Figure 4. Absorption spectra for $4.0 \cdot 10^{-5}$ mol·L^{-1} methyl red in water−ethanol (80/20) and in the presence of different concentrations of β-CD at pH 5.2. The concentrations of β-CD are: (a) 0.0, (b) $4.0 \cdot 10^{-4}$ mol·L^{-1}, (c) $1.0 \cdot 10^{-3}$ mol·L^{-1}, (d) $2.0 \cdot 10^{-3}$ mol·L^{-1}, and (e) $4.8 \cdot 10^{-3}$ mol·L^{-1}.

As Figure 4 shows, in the mixed solvent containing 20 % (v/v) ethanol the absorbance change in the presence of β-CD is less than that in pure water solvent with the same conditions. The presence of ethanol as a hydrophobic cosolvent decreases the stability of inclusion complexes and then causes less absorbance changes.

The number of absorptive species may also be determined by applying PCA on the data matrix with the absorption spectra of $4.0 \cdot 10^{-5}$ mol·L^{-1} methyl red in the presence of $1.4 \cdot 10^{-3}$

Table 1. Results of PCA on Experimental Data for Methyl Red ($4\cdot10^{-5}$ mol·L^{-1} in the Presence of $1.4\cdot10^{-3}$ mol·L^{-1} β-CD at Different Values of pH)

i	g_i	g_i/g_{i+1}	R.S.D.
1	27.223	2.6129	0.066733
2	10.419	19.347	0.02248
3	0.53851	1.6936	0.017649
4	0.31797	1.6169	0.013393
5	0.19665	3.6817	0.008832
6	0.053414		

Table 2. $K'_{a,c}$ Values for Methyl Red in the Presence of Different Concentrations of β-CD and Different Water−Ethanol Mixtures at Constant Ionic Strength of 0.15 mol·L^{-1}

β-CD	water		water−ethanol (80/20)		water−ethanol (65/35)	
(mol·L^{-1})	$K'_{a,c}$	R.S.D.	$K'_{a,c}$	R.S.D.	$K'_{a,c}$	R.S.D.
0	$1.25\cdot10^{-5}$	0.0342	$3.99\cdot10^{-6}$	0.0168	$2.81\cdot10^{-6}$	0.0129
$2.0\cdot10^{-4}$	$1.41\cdot10^{-5}$	0.0244	$4.36\cdot10^{-6}$	0.0152	$2.81\cdot10^{-6}$	0.0188
$4.0\cdot10^{-4}$	$1.58\cdot10^{-5}$	0.0162	$4.57\cdot10^{-6}$	0.0187	$2.88\cdot10^{-6}$	0.0129
$1.0\cdot10^{-3}$	$1.77\cdot10^{-5}$	0.0110	$5.01\cdot10^{-6}$	0.0191	$2.95\cdot10^{-6}$	0.0160
$1.4\cdot10^{-3}$	$1.99\cdot10^{-5}$	0.0199	$5.24\cdot10^{-6}$	0.0142	$3.01\cdot10^{-6}$	0.0166
$2.0\cdot10^{-3}$	$2.08\cdot10^{-5}$	0.0127	$5.49\cdot10^{-6}$	0.0131	$3.16\cdot10^{-6}$	0.0157
$2.6\cdot10^{-3}$	$2.23\cdot10^{-5}$	0.0105	$5.62\cdot10^{-6}$	0.0165	$3.16\cdot10^{-6}$	0.0169
$3.2\cdot10^{-3}$	$2.29\cdot10^{-5}$	0.0199	$5.75\cdot10^{-6}$	0.0105	$3.31\cdot10^{-6}$	0.0172
$3.8\cdot10^{-3}$	$2.34\cdot10^{-5}$	0.0105	$6.02\cdot10^{-6}$	0.0134	$3.46\cdot10^{-6}$	0.0187
$4.8\cdot10^{-3}$	$2.39\cdot10^{-5}$	0.0183	$6.30\cdot10^{-6}$	0.0121	$3.54\cdot10^{-6}$	0.0212

mol·L^{-1} β-CD in water−ethanol (80/20, v/v) at different pH values. The results are presented in Table 1.

The ratio of consecutive eigenvalues reaches a maximum at $i = 2$ that indicates there exist two absorptive components in the considered acid−base equilibrium system. The conditional acidity constant $K'_{a,c}$ is optimized by the proposed method. A narrow minimum is observed in the R.S.D. curve shown in Figure 5 which indicates the optimum value of p$K'_{a,c}$, and the optimal set solution is $K'_{a,c} = 5.24\cdot10^{-6}$ with R.S.D. = 0.0142.

Conditional acidity constant values for methyl red in the absence and presence of different contents of ethanol and at different cyclodextrin concentration were obtained. The results are given in Table 2. As shown in this table, the acidity constant of methyl red decreased as the content of the ethanol increased. Also its conditional acidity constant increased by increasing the concentration of β-CD. Consequently, the increase in the acidity constants as a function of β-CD is due to more stabilization of the basic form of methyl red in the presence of β-CD than its acidic form.

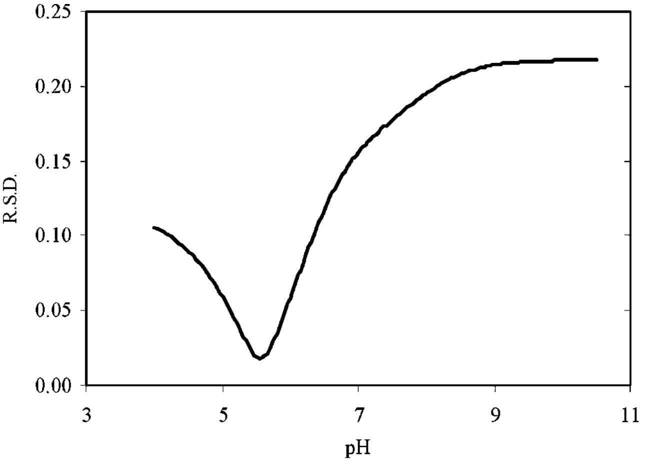

Figure 5. Relationship between R.S.D. and p$K_{a,c}$ values for $4\cdot10^{-5}$ mol·L^{-1} methyl red in the presence of $1.4\cdot10^{-3}$ mol·L^{-1} β-CD at different pH values in water−ethanol (80/20) solution.

The conditional acidity constant values for neutral red, 4-nitrophenol, and 4-nitrocatechol in various water−ethanol mixtures and in the presence of different concentrations of β-CD were also obtained by the same method described for methyl red. The conditional acidity constants and R.S.D. obtained by RAFA are listed in Table 3.

The effects of inclusion of neutral red, 4-nitrophenol, and 4-nitrocatechol in β-CD on their acid−base equilibria in aqueous solutions was discussed in our previous paper.[8] The following results were obtained by perusal of Tables 2 and 3: for 4-nitrophenol and 4-nitrocatechol by increasing the ethanol content of solution, their acidity constants decrease as for methyl red. But the acidity constant of neutral red increases with increasing ethanol content. Methyl red, 4-nitrocatechol, and 4-nitrophenol are HA type acids, and the solvating ability and relative permittivity play a fundamental role in dissociation reactions.[17] The energy required to separate the opposite charges of an acid in a solvent is inversely proportional to the relative permittivity of the solvent. Consequently, decreasing the permittivity of solution decreases their acidity constant. This explains that their K_a value decreases as the content of ethanol in the mixture increases. The acidic form of neutral red has a positive charge (BH^+), and its basic form is neutral. On the basis of

Table 3. $K'_{a,c}$ Values for 4-Nitrophenol, 4-Nitrocatechol, and Neutral Red in the Presence of Different Concentrations of β-CD and Different Water−Ethanol Mixtures at Constant Ionic Strength of 0.15 mol·L^{-1}

	β-CD	water[a]		water−ethanol (80/20)		water−ethanol (65/35)	
compound	(mol·L^{-1})	$K'_{a,c}$	R.S.D.	$K'_{a,c}$	R.S.D.	$K'_{a,c}$	R.S.D.
4-nitrocatecohol	0	$3.01\cdot10^{-7}$	0.0071	$1.77\cdot10^{-7}$	0.0041	$1.44\cdot10^{-7}$	0.0054
	$8.0\cdot10^{-4}$	$3.89\cdot10^{-7}$	0.0077	$1.99\cdot10^{-7}$	0.0059	$1.58\cdot10^{-7}$	0.0079
	$1.6\cdot10^{-3}$	$4.36\cdot10^{-7}$	0.0077	$2.13\cdot10^{-7}$	0.0038	$1.73\cdot10^{-7}$	0.0076
	$3.2\cdot10^{-3}$	$5.12\cdot10^{-7}$	0.0091	$2.23\cdot10^{-7}$	0.0038	$1.77\cdot10^{-7}$	0.0076
	$4.8\cdot10^{-3}$	$5.75\cdot10^{-7}$	0.0082	$2.51\cdot10^{-7}$	0.0046	$1.81\cdot10^{-7}$	0.0074
	$7.2\cdot10^{-3}$	$6.16\cdot10^{-7}$	0.0093	$2.63\cdot10^{-7}$	0.0058	$2.08\cdot10^{-7}$	0.0056
4-nitrophenol	0	$1.25\cdot10^{-7}$	0.0105	$1.12\cdot10^{-7}$	0.0053	$1.00\cdot10^{-7}$	0.0049
	$8.0\cdot10^{-4}$	$1.62\cdot10^{-7}$	0.0264	$1.25\cdot10^{-7}$	0.0052	$1.12\cdot10^{-7}$	0.0057
	$1.6\cdot10^{-3}$	$1.90\cdot10^{-7}$	0.0224	$1.31\cdot10^{-7}$	0.0051	$1.25\cdot10^{-7}$	0.0052
	$3.2\cdot10^{-3}$	$2.23\cdot10^{-7}$	0.0131	$1.58\cdot10^{-7}$	0.0064	$1.41\cdot10^{-7}$	0.0064
	$4.8\cdot10^{-3}$	$2.45\cdot10^{-7}$	0.0123	$1.73\cdot10^{-7}$	0.0056	$1.51\cdot10^{-7}$	0.0067
	$7.2\cdot10^{-3}$	$2.51\cdot10^{-7}$	0.0088	$1.81\cdot10^{-7}$	0.0072	$1.58\cdot10^{-7}$	0.0068
neutral red	0	$2.88\cdot10^{-7}$	0.0338	$3.31\cdot10^{-7}$	0.0105	$7.94\cdot10^{-7}$	0.0112
	$8.0\cdot10^{-4}$	$3.8\cdot10^{-7}$	0.0264	$3.55\cdot10^{-7}$	0.0093	$8.51\cdot10^{-7}$	0.0143
	$1.6\cdot10^{-3}$	$4.57\cdot10^{-7}$	0.0224	$4.17\cdot10^{-7}$	0.0112	$8.91\cdot10^{-7}$	0.0133
	$3.2\cdot10^{-3}$	$5.75\cdot10^{-7}$	0.0131	$4.79\cdot10^{-7}$	0.0128	$1.00\cdot10^{-6}$	0.0172
	$4.8\cdot10^{-3}$	$6.92\cdot10^{-7}$	0.0123	$5.01\cdot10^{-7}$	0.0134	$1.00\cdot10^{-6}$	0.0157
	$7.2\cdot10^{-3}$	$7.24\cdot10^{-7}$	0.0088	$5.25\cdot10^{-7}$	0.0107	$1.00\cdot10^{-6}$	0.0116

[a] Data taken from ref 8.

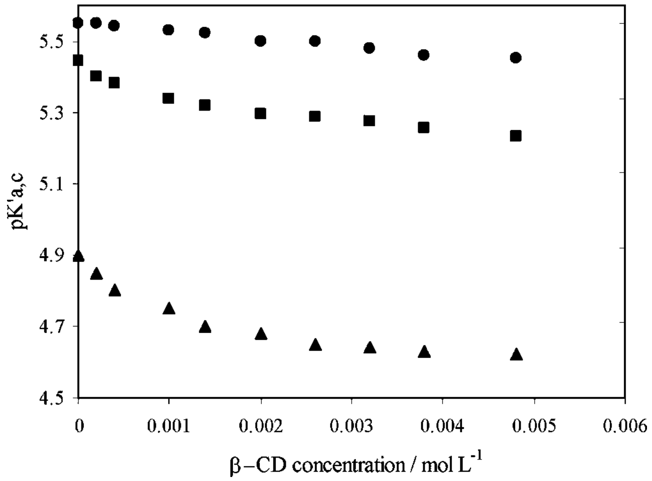

Figure 6. Plots of $pK_{a,c}$ for methyl red as a function of concentrations of β-CD in ▲, water; ■, 80/20 water−ethanol; and ●, 65/35 water−ethanol solutions.

Table 4. Stability Constants for Inclusion Complexes of Methyl Red, 4-Nitrophenol, and 4-Nitrocatechol with β-CD in Water and Water−Ethanol Solutions at Constant Ionic Strength of 0.15 mol·L^{-1}

compound	solvent	$K_{f,HA}$ (M^{-1})	$K_{f,A-}$ (M^{-1})
methyl red	water	581	1300
	water−ethanol (80/20)	545	932
	water−ethanol (65/35)	405	495
4-nitrophenol	water	353[a]	861
	water−ethanol (80/20)	295	673
	water−ethanol (65/35)	228	440
4-nitrocatechol	water	239[a]	634
	water−ethanol (80/20)	155	354
	water−ethanol (65/35)	142	256

[a] Data taken from ref 8.

this concept, addition of ethanol will drive the equilibrium to produce the neutral basic form and should increase the acidity constant.[18] For more details, variations of the conditional acidity constants were plotted toward β-cyclodextrin concentration in various solvent conditions (Figure 6).

Nonlinear least-squares fits of the experimental α values in various concentrations of CD yielded the calculated values of $K_{f,HA}$ and $K_{f,A-}$ for these systems. The results are presented in Table 4.

We observed that the complexation of both acidic and basic forms of the investigated compounds with β-CD takes place. Preferential inclusion of the acid form causes a decrease in the conditional acidity constant, and preferential inclusion of the basic form causes an increase in the conditional acidity constant. Generally, for all the investigated compounds, the stability of the inclusion complexes decreased with increasing amounts of ethanol in the solvent mixture. As mentioned above, addition of ethanol lowers the relative permittivity of the aqueous medium and changes its solvophobic characteristics. On one hand, indication of conformational changes experienced by the macrocycle is induced, while on the other hand, the guest molecules are stabilized by the different solvent media. Also, the possible encapsulation of the alcohol molecule by the cyclodextrin may partially render formation of the indicators complex.[19]

Conclusions

RAFA is proposed as an efficient chemometrics method for complete analysis of acid−base equilibria of some indicators in water and mixed ethanol−water solutions. The method was tested with simulated data. Apparent acidity constants of methyl red, 4-nitrophenol, 4-nitrocatechol, and neutral red were obtained by RAFA. Their formation constants with β-cyclodextrin at constant ionic strength were obtained by curve fitting. The dissociation constants of these indicators are primarily governed by electrostatic factors in mixed solvents. Addition of ethanol with less permittivity than water affected the acidity constants by stabilization of the neutral form of the analytes. Also, addition of ethanol as an organic solvent decreased the stability constants of the inclusion complexes of β-cyclodextrin with both acidic and basic forms of the investigated compounds.

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