Spectrophotometric Determination of Acidity Constants of Alizarine Red S in Mixed Aqueous−Organic Solvents

Jahanbakhsh Ghasemi,*,† Shahram Lotfi,‡ Mahbobeh Safaeian,‡ Ali Niazi,§ Mohammad Mazloum Ardakani,‡ and Mohammad Noroozi|

Department of Chemistry, Faculty of Sciences, Razi University, Kermanshah, Iran, Department of Chemistry, Payame Noor University, Ardakan Unit, Ardakan, Iran, Department of Chemistry, Faculty of Sciences, Azad University of Arak, Arak, Iran, and Research Institute of Petroleum Industry, Kermanshah Research Center of Oil and Engineering, Kermanshah, Iran

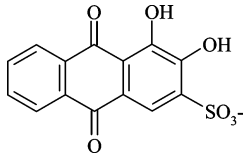
The effect of different organic−water mixtures on the acidity constants of alizarine red s (9,10-dihydro-3,4-dihydroxy-9,10-dioxo-2-anthracenesulfonic acid) were determined at 25 °C and an ionic strength of 0.1 M by a multiwavelength spectrophotometric method. Two pK_a values for the -OH derivatives were determined. The organic solvents used were the amphiprotic (methanol, ethanol), dipolar aprotic (dimethyl sulfoxide), and low basic aprotic (acetonitrile). To evaluate the pH absorbance data, a resolution method based on the combination of soft- and hard-modeling is applied. The acidity constants of all related equilibria are estimated using the whole spectral fitting of the collected data using an established factor analysis model. The data analysis program DATAN was applied for determination of acidity constants. Generally, the pK_a values increase with an increase in the content of the organic solvent. This behavior can be accounted for in terms of the high stabilization of both the nonprotonated and the ionic forms of this compound by dispersion forces rather than by hydrogen bonding. There are linear relationships between acidity constants and the mole fraction of different solvents in the mixtures. The effect of solvent properties on acid−base behavior is discussed.

Introduction

Acid dissociation constants are important parameters to indicate the extent of ionization of molecules in solution at different pH values. The acidity constants of organic reagents play a fundamental role in many analytical procedures such as acid−base titrations, solvent extraction, complex formation, and ion transport. It has been shown that the acid−base properties affect the toxicity, chromatographic retention behavior, and pharmaceutical properties of organic acids and bases. Much of the theoretical foundation of modern organic chemistry is based on the observation of the effects on acid−base equilibrium of changing molecular structure.[1−5]

The widespread application of anthraquinone compounds as dyes, acid−base, drugs, metallochrome indicators, or histological stains have attracted the attention of many researchers to study their acid−base and complex formation properties. However, the literature lacks studies on the acid−base properties or medium effects on the acid dissociation constants of these compounds, which are thought to be of special interest owing to their biological and therapeutical importance.[6−10] In continuation of our studies on the acid−base properties of these compounds,[1,11] we have investigated the medium effect on the ionization constants of alizarin red s (see Scheme 1 for structure) as a good representation of anthraquinone derivatives by the study of the electronic spectra of the compound in aqueous buffer solutions containing varying proportions of organic solvents of different polarities, such as methanol, ethanol, acetonitrile, and dimethyl sulfoxide. The p*K*a values have been determined and discussed in terms of solvent characteristics.

Scheme 1. Chemical Structure of Alizarine Red S



The solvation of a solute in a mixed solvent is much more complex than the solvation in a pure single solvent, and the literature offers several theories and models for this process.[12] In general, they agree that, when a solute is dissolved in a mixed solvent, the specific solvation effects determine that the proportion of the solvents in the solute's sphere of solvation is different from the proportion in the bulk solvent. The solute interacts more strongly with one or more solvents of the mixture, and it is preferentially solvated by these solvents. Solute properties, such as the pK_a values, depend on the composition and properties of this solvation sphere; therefore, they are very sensitive to preferential solvation.

The spectroscopic instrumentation used today, however, almost invariably has the capacity to collect data across a full spectral range. Using a single or a few wavelengths discards most of the information in the collected spectra and requires both the presence and knowledge of such suitable wavelengths. However, in many cases, the spectral responses of components overlap, and analysis is no longer straightforward.[5,13] The determination of association constants using spectroscopic measurements is commonly accomplished using the method of Benesi−Hildebrand.[14,15] This analysis requires that the concentration of one of the associating species be kept much lower than the other, and it assumes that the dissociated species do

* Corresponding author. Tel: +98 831 835 8077. Fax: +98 831 836 9572. E-mail: jahan.ghasemi@gmail.com.
† Razi University.
‡ Payame Noor University.
§ Azad University of Arak.
| Kermanshah Research Center of Oil and Engineering.

10.1021/je050535d CCC: $30.25
Published on Web 07/06/2006

not contribute significantly to the measured analytical signal. The single-point measurements are usually made at the edge of an absorption band, where the spectral overlap is least. However, here the spectral responses are much lower than at the absorption maximum, the noise level may be considerable, and the association constants determined by the Benesi−Hildebrand method are accompanied with greater systematic errors.[16]

Using chemometric methods, whole spectra can be analyzed, thereby utilizing all spectral information.[17,18] The approach is superior to any single-point measurement since several hundred data points per spectrum can be treated simultaneously.[19] The predefined model, known as hard-modeling analysis, cannot be applied if crucial information is missing. Soft-modeling or model free approaches are based on much more general prerequisites, such as positive molar absorbance, positive concentration of all species, unimodality of concentration profiles, and closure (concentration of all species are the same for all solutions). Naturally, if the strengths of hard-modeling and soft-modeling methodologies are combined, a much more powerful method of data analysis can be expected.[20−26]

Data analysis, carried out by the DATAN package that was developed by Kubista group[13,26] and is called a physical constraints approach, provides a unique solution by requiring that the calculating concentrations obey an assumed equilibrium expression. It has been demonstrated by application to the determination of the acidity constants of two and four protolytic forms of fluorescein. A possible advantage of the Kubista et al.[13] method is that it mixes a soft-modeling approach with a hard-modeling approach. This might be a better and more general strategy, since it can handle different situations, with only a partial knowledge of the chemistry of the system. The physical constraints method calculates spectral profiles, concentrations, and equilibrium constants by utilizing equilibrium expressions that are related to the components. The theory and application of the physical constraints method has been discussed by Kubista et al. in several papers,[27−36] and it is briefly presented below.

Spectra of alizarin red s at different pH values are digitized and arranged in a data matrix ***A***, which is decomposed into an orthonormal basis set by NIPALS or any equivalent method:[13]

$$\mathbf{A} = \mathbf{TP}' + \mathbf{E} \approx \mathbf{TP}' = \sum_{i=1}^{r} \mathbf{t}_i \mathbf{p}_i \tag{1}$$

where the orthogonal target vectors $\mathbf{t}_i$ and orthonormal projection vectors $\mathbf{p}_i$ are mathematical constructs that cannot be directly related to component spectra and concentrations; r is the number of independent spectroscopic components, which corresponds to the number of light-absorbing chemical species. It is determined by visual inspection of the **t** and **p′** vectors or by performing statistical methods.[27,30] **E** is an error matrix. By assuming linear responses, the spectra in matrix **A** are linear combinations of the concentrations, C, and spectral responses, V, of the chemical components:

$$\mathbf{A} = CV + \mathbf{E} \approx CV \tag{2}$$

If the spectral profiles of the components are known, the concentration of each component can easily be calculated, for example, by least squares minimization. If standards are not available, the common belief has been that the components spectral responses cannot be separated, which precludes their identification. This is due to ambiguity in determining the rotation matrix, **R**, in the following equations; from eqs 1 and 2 it follows that there is a square matrix **R** ($r \times r$) that satisfies the following:

$$\mathbf{T} = C\mathbf{R} \tag{3a}$$

$$\mathbf{P} = \mathbf{R}^{-1}V \tag{3b}$$

Since $\mathbf{A} = CV = C(\mathbf{R}\mathbf{R}^{-1})V = (C\mathbf{R})(\mathbf{R}^{-1}V) = \mathbf{TP}'$. If **R** can be determined, the spectral responses V and concentrations C of the components can be calculated from the target **T** and projection **P′** matrices:

$$C = \mathbf{TR}^{-1} \tag{4a}$$

$$V = \mathbf{RP}' \tag{4b}$$

The thermodynamic expression that describes the components concentration is the main constraint used to determine **R**, from which thermodynamic parameters and components spectral responses and concentration are calculated. Therefore, the strategy for determining the rotation matrix **R** is as follows. Concentrations of the chemical species are calculated from the equilibrium expressions for various trial values of the equilibrium constants and are fitted to the calculated target vectors according to eq 3a. The accuracy of this fit depends crucially on the trial values of the equilibrium constants, and the best fit determines their values and the elements of matrix **R**.

In this study, the physical constraints approach has been applied to determine the acidity constants of alizarine red s in pure water and in different binary organic solvent−water mixtures by spectrophotometric titration at 25 °C and an ionic strength of 0.1 M. The analysis is readily performed with the DATAN program[37] that was developed by the Kubista group.

Experimental Section

Materials. Alizarine red s, ethanol, methanol, acetonitrile, dimethyl sulfoxide, hydrochloric acid, sodium hydroxide, and potassium nitrate were analytical grade commercial products from Merck. These reagents were used without further purification. Standard stock solutions of 1.0×10^{-3} M alizarine red s were prepared by dissolving appropriate amounts of alizarine red s in water. All the solutions were prepared in deionized water.

Instrumentation and Software. A Scinco (S-2100) spectrophotometer controlled by a computer and equipped with a 1-cm path length quartz cell was used for UV−vis spectra acquisition. Spectra were acquired between 315 and 650 nm (1 nm resolution). The pH values were measured by a Metrohm CH-9101 pH meter furnished with combined calomel Ag/AgCl electrode. To precalibrate the pH meter in the various binary organic + water mixtures used, 0.01 M solutions of oxalate and succinate buffers were employed. Then to calibrate the pH meter according to the concentration of H^+ some strong acid−base titrations were performed. As the proton concentrations driving strong acid−strong base titrations can be readily calculated, the concentration pH value($p_cH = -\log[H^+]$) is related to the operational pH. The details of the electrode calibration in partially aqueous solutions media are presented elsewhere.[38] The data were treated in an AMD 2000 XP (256 MB RAM) microcomputer using the DATAN package.

Spectrophotometric Measurements. For the alizarine red s (3×10^{-5} M) in binary mixture titrations, absorption spectra were measured with a titration setup consisting of a computer interfaced to a spectrophotometer. After each pH adjustment, solution is transferred into the cuvette, and the absorption spectra are recorded. Ionic strength was maintained at 0.1 M by adding appropriate amounts of KNO_3. The pH values in organic solvent

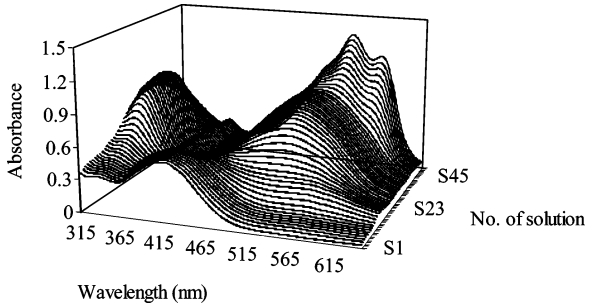


Figure 1. Absorption spectra of alizarine red s in pure water at 0.1 M KNO_3 at different pH values.

+ water mixtures were corrected using the equation $pH^* = pH(R) - \delta$, where pH^* is the corrected reading and $pH(R)$ is the pH meter reading obtained in a partially aqueous organic solvent, determined by Douheret.[39,40] All measurements were carried out at the temperature (25.0 ± 0.5) °C.

Results and Discussion

The absorption spectra of alizarine red s in binary solvent mixtures at various pH values and in the interval (315 to 650) nm were recorded. Sample spectra of alizarine red s at different pH values in pure water with the pH ranging from 1.9 to 12.8 and 60 % (w/v) of the organic solvents (methanol, ethanol, acetonitrile, and dimethyl sulfoxide with the pH ranging from 2.9 to 12.4, from 1.9 to 12.8, from 2.3 to 12.6, and from 2.6 to 12.7) to water at 0.1 M KNO_3 are shown in Figures 1 and 2, respectively.

The decisive and very important step in the data analysis of solution chemical equilibria studies is to determine the number of light absorbing species in the equilibrium system. The number of light absorbing species in the absorption spectra is obtained by different approximate methods requiring no knowledge of the instrumental error of the absorbance data. Many of these methods are empirical functions. Singular value decomposition analysis performed on all absorption data matrices obtained at various pH values for alizarine red s gives the number of components that best represent the system. According to eigenvalue results, three components are present in the systems studied in this work. The existence of the three significant factors are also supported by the statistical indicators, which were introduced by Elbergali et al.[30] These factors could be attributed to the two dissociation equilibria of a diprotic acid such alizarine red s.

The pK_a values of alizarine red s were investigated in different methanol, ethanol, acetonitrile, and dimethyl sulfoxide + water binary mixtures spectrophotometrically at 25 °C and an ionic strength of 0.1 M. Acidity constants of alizarine red s in several mixtures were evaluated using the computer program DATAN and the corresponding spectral absorption−pH data. From inspection of the experimental spectra, it is hard to guess even the number of protolytic species involved. The three calculated most significant projection vectors with clear spectral features (as compared to noise) indicate the presence of three spectroscopically distinguishable components. Their profiles or shapes show some order of ambiguity, (i.e., they are clearly physically meaningless and cannot be directly related to the spectral response of the three protolytic forms). After rigorous curve resolution computational steps according to combination of hard- and soft-modeling, the outputs of the program are pK_a values and their standard deviation (derived from the error analysis plot of the program), number of principal components, projection vectors (loadings), concentration distribution diagrams, and pure spectrum of each assumed species.

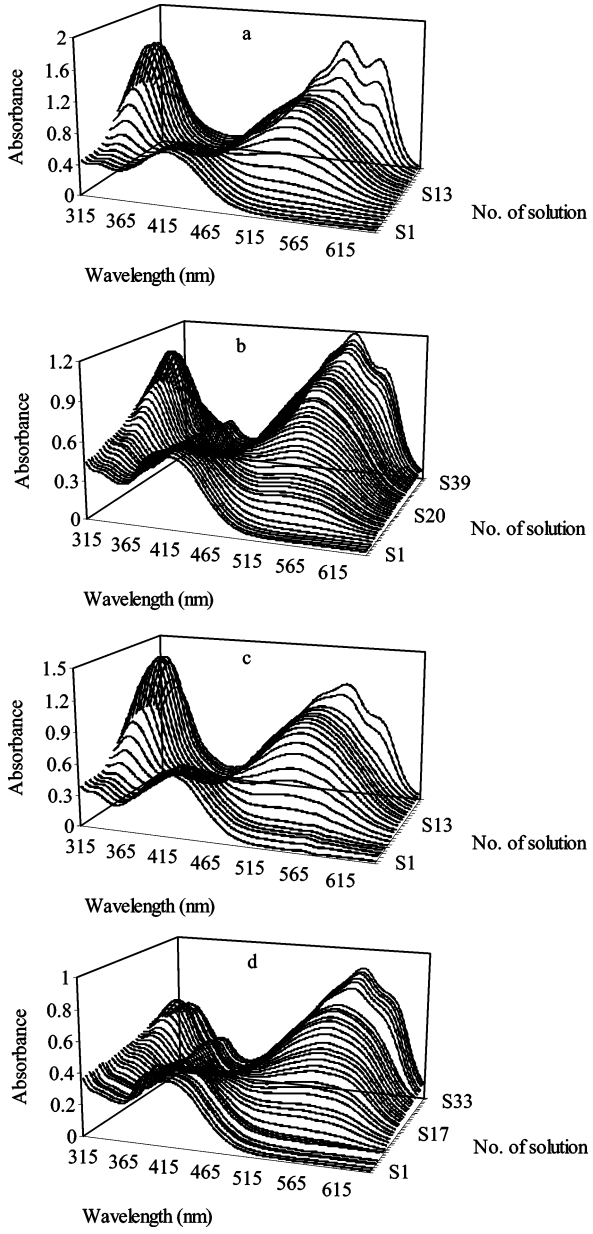


Figure 2. Absorption spectra of alizarine red s in (a) 60 wt % methanol to water, (b) 60 wt % ethanol to water, (c) 60 wt % acethonitrile to water, and (d) 60 wt % dimethylsulfoxide to water at 0.1 M KNO_3 at different pH values.

The pK_a values obtained are listed in Table 1. The previous reported values of acidity constants are mainly in pure water, in mixtures of dioxane with water, and in micellar media solution.[41,42] The obtained values in pure water are in good agreement with previous values,[41] which are listed in Table 1 for comparison. The differences observed between the pK_a values are not only in the experimental errors margins but also due to different computational strategy of the univariate methods and of newer chemometrics based methods. The way of noise or measurement errors treatment on the absorption spectra are performed in a multivariate sense, which use the whole spectral domain, reduce considerably the level of noise, and result in more precise final information. So the acidity constants obtained are more reliable and precise than previous methods. The pK_a values correspond to the pH-dependent variation of absorption spectra in all solvents mixtures. One of the very important outputs of the DATAN program is the calculated spectrum of different forms of alizarine red s in each solvent mixture. Sample

Table 1. Acidity Constants of Alizarine Red S in Different Organic Solvents + Water Mixtures at 25 °C and Ionic Strength of 0.1 M by the DATAN Program

	previous reports		methanol		ethanol		acetonitrile		dimethyl sulfoxide	
wt %	pK_{a1}	pK_{a2}	pK_{a1}	pK_{a2}	pK_{a1}	pK_{a2}	pK_{a1}	pK_{a2}	pK_{a1}	pK_{a2}
0	5.50[a]	11.00[a]	5.55 ± 0.02	11.50 ± 0.08						
	5.49[a]	10.85[a]								
	6.10[b]	10.80[b]								
10			6.16 ± 0.04	11.71 ± 0.08	5.94 ± 0.01	11.55 ± 0.06	6.14 ± 0.04	11.75 ± 0.07	6.04 ± 0.02	11.46 ± 0.08
20			6.34 ± 0.03	11.93 ± 0.09	5.97 ± 0.02	11.74 ± 0.05	6.26 ± 0.04	12.05 ± 0.09	6.11 ± 0.03	11.65 ± 0.07
30			6.65 ± 0.04	12.20 ± 0.08	5.86 ± 0.04	12.58 ± 0.08	6.53 ± 0.05	12.32 ± 0.09	6.44 ± 0.03	11.84 ± 0.09
40			6.75 ± 0.05	12.34 ± 0.08	6.52 ± 0.03	12.68 ± 0.07	6.91 ± 0.04	12.73 ± 0.07	6.55 ± 0.04	11.97 ± 0.08
50			6.98 ± 0.06	12.5 ± 0.1	6.33 ± 0.04	12.79 ± 0.08	7.26 ± 0.03	12.93 ± 0.08	6.53 ± 0.05	12.27 ± 0.08
60			7.06 ± 0.05	12.6 ± 0.1	6.63 ± 0.05	12.99 ± 0.09	7.46 ± 0.06	13.0 ± 0.1	6.85 ± 0.05	12.4 ± 0.1

[a] Ref 34 (pure water). [b] Ref 35 (75 % dioxane).

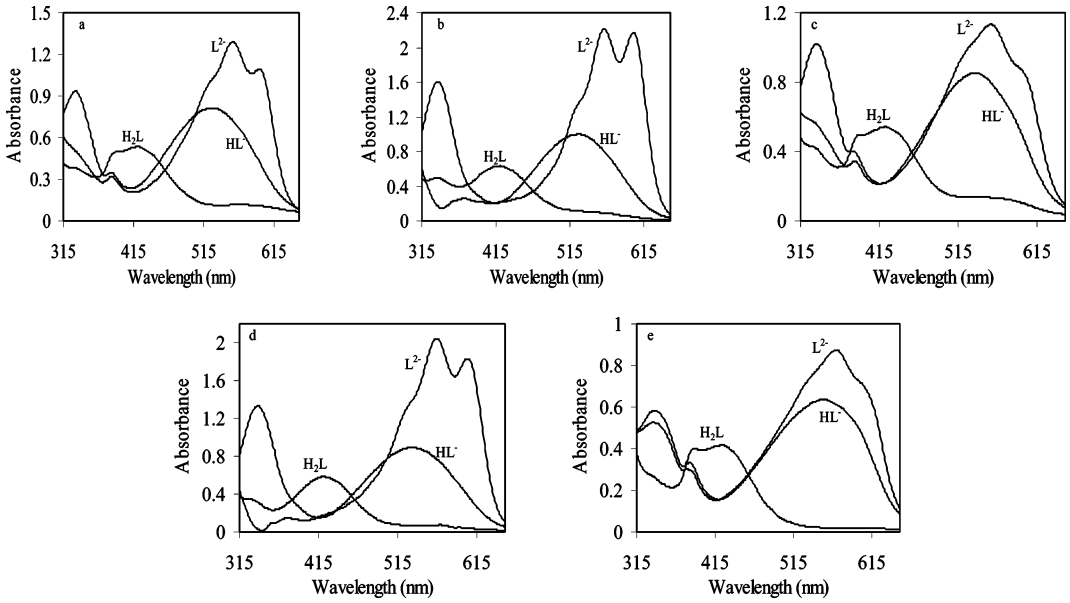


Figure 3. Components absorption spectra of different form of alizarine red s in (a) pure water, (b) 60 wt % methanol to water, (c) 60 wt % ethanol to water, (d) 60 wt % acethonitrile to water, and (e) 60 wt % dimethylsulfoxide to water at 0.1 M KNO_3 at different pH values.

spectra of the calculated pure spectral profiles of all species in water and different organic solvents/water mixtures are shown in Figure 3. As the mole fraction of organic solvents increased, the absorption intensity changes differently for each species of alizarin red s. It is interesting to note that the nature and the composition of the solvent have a fundamental effect on each pure spectrum. As is clear from Figure 3, this effect is more for L^{2-} and H_2L than HL^-. The spectrum of the L^{2-} species has a λ_{max} at 555 nm which show a splitting pattern in high weight percent of methanol and acetonitrile. The splitting of the absorption peak at λ_{max} of L^{2-} is more obvious than the effect on the other species. This can be described using the nonelectrostatic (H-bonding) property of the stabilization and/or destabilization of the ground and excited states of the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions. The appearance and disappearance of some shoulder and absorption peaks of each species is related to the type and mass percent of the organic solvent.

Increasing the mole fraction of solvents in the medium leads to a decrease in the acid ionization constants for alizarine red s. The acid ionization constants in a pure aqueous medium ($K_{a(w)}$) is related to that in a partial aqueous medium ($K_{a(s)}$) by the relation:[42]

$$K_{a(w)} = K_{a(s)}\left(\frac{\gamma_{A^-}\gamma_{H^+}}{\gamma_{HA}}\right)$$

where γ is the activity coefficient of the respective species in a partial aqueous medium relative to that in pure water. The variations of electrostatic effects resulting from the change in the relative permittivity of the medium operate on the activity coefficient of all charged species.[42] Thus, one can expect that increasing the content of the organic solvent in the medium will increase the activity coefficient of both the H^+ and conjugate base A^-. Consequently, according to above equation, increasing the mole fraction of the solvents should decrease the acid ionization constants (i.e., pK_a values increase).

As discussed above, this indicates that the acid ionization constants of alizarine red s obtained in various aqueous mixture media of methanol, ethanol, acetonitrile, and dimethyl sulfoxide are governed by electrostatic effects. Acidity constants of two steps of dissociation of alizarine red s decrease with increasing mole fraction of the solvents in the mixed binary solvents. It has been shown that the solvating ability[42] (as expressed by the Gutmann donicity scale) and dielectric constant of the solvent play a fundamental role in dissociation reactions. Water is a solvent of high solvating ability (i.e., donor number DN = 33,[22] dielectric constant ϵ = 87.3), which can dissociate the acid and stabilize the produced anion and hydrogen ion. Thus, it is expected that addition of methanol (DN = 19, ϵ = 32.6), ethanol (DN = 15, ϵ = 24.3), acetonitrile (DN = 14, ϵ = 36), and dimethyl sulfoxide (DN = 26.5, ϵ = 46.6) with lower donor numbers and dielectric constants relative to water decrease the extent of interaction between the acid anion and proton with

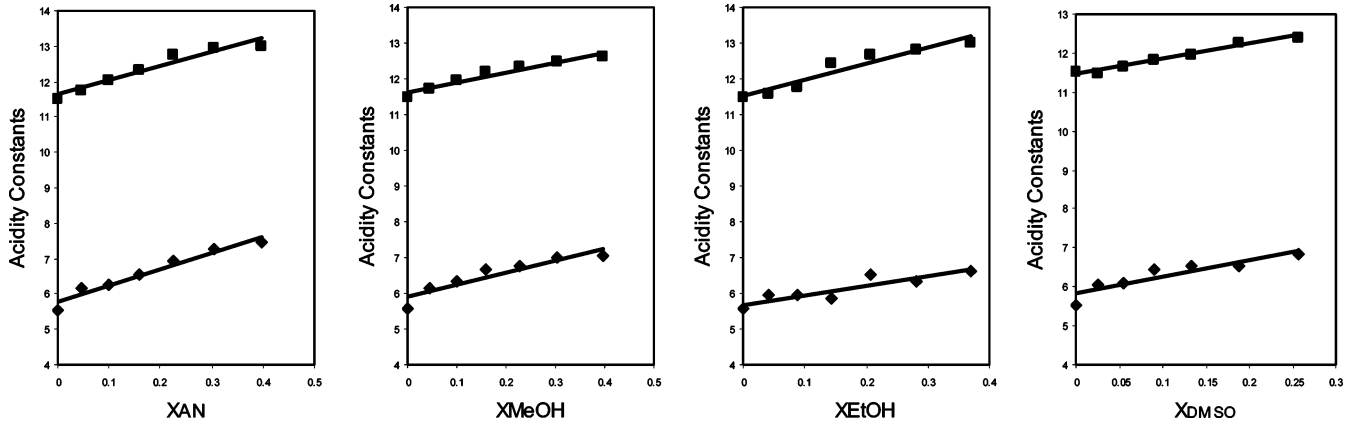


Figure 4. Variation of acidity constants values of alizarine red s with mole of different organic solvents, upper line pK_{a1} and lower line pK_{a2}.

solvent, and this decreases the acidity constants of alizarine red s.

It is interesting to note that there is actually a linear relationship between the pK_a of two dissociation steps and the mole fraction of different solvents ($X_{Solvents}$) in the binary mixed solvents used in Figure 4. The same trend has already been reported for various organic molecules in different solvent mixtures.[21,22,43−45] It has been reasonably assumed that preferential solvation of the charged particles by water is mainly responsible for such a monotonic dependence of acidity constants of the alizarine red s on the solvent composition.

It was recognized that solvent effects such as hydrogen bonding and solvent basicity as well as dispersion forces and proton−solvent interactions exert a profound influence on the ionization process of weak acids in the presence of organic solvents such as methanol, ethanol, acetonitrile, and dimethyl sulfoxide. The effective density of dispersion centers in the organic solvents used are higher than in pure water.[40] Accordingly, one can expect a higher stabilization of the conjugate base A^- of each step of ionization by dispersion forces, which are established between the delocalized oscillator dipole of the solvent. Furthermore, the proton is expected to be highly stabilized in aqueous mixtures by its interaction with the organic solvent and water molecules (proton−solvent interaction) as compared with water molecules alone. Consequently, both A^- and H^+ will be highly stabilized upon the increasing of the mole fraction of the organic solvents in the aqueous medium, that is, γ_{A^-} and γ_{H^+} are decreased. Thus, the acid ionization constants of the studied alizarine red s would increase (pK_a decrease) with an increase of the studied solvents content in the medium. However, this is not the case, as is evident from the data listed in Table 1. Therefore, one can conclude that both the dispersion forces and proton−solvent interaction effects do not have a significant role in the ionization processes of the studied alizarine red s compound.

On the other hand, water molecules are characterized by a high tendency to donate hydrogen bonds as compared with other solvent molecules. Therefore, the conjugate base A^- is expected to be less stabilized by hydrogen-bonding interaction with the studied solvent molecules as the mole fraction of solvents is increased (i.e., γ_{A^-} increase). This will tend to increase the pK_a values of all steps in the alizarine red s system. Accordingly, the observed increase in the pK_a values of alizarine red s upon an increasing mole fraction of the solvents in aqueous mixtures can be described, in addition to the electrostatic effect, to the hydrogen-bonding interaction between the conjugate base A^- and the organic solvent molecules.

Conclusions

In this study, we distinguish the behavior of acidity constants of alizarine red s in pure water, water−methanol, water−ethanol, water−acetonitrile, and water−dimethyl sulfoxide systems at 25 °C and an ionic strength of 0.1 M that has been studied by a multiwavelength spectrophotometric method. Results show that the pK_a values of alizarine red s are influenced by the percentages of organic solvents such as methanol, ethanol, acetonitrile, and dimethyl sulfoxide added to the solution of this reagent. DATAN is a useful tool for resolution of the different species present in equilibrium systems. By using this method and without any prior knowledge about the system, we can obtain concentration profiles and pure spectra from the experimental data. The effect of solvent properties on acid−base behavior is discussed. In conclusion, this indicates that the acid ionization constants of alizarine red s obtained in various aqueous mixture media of organic solvents is governed by electrostatic effects.

Literature Cited

(1) Almasifar, D.; Forghaniha, F.; Khojasteh, Z.; Ghasemi, J.; Sharghi, H.; Shamsipur, M. Spectrophotometric determination of acidity constants of some recently synthesized antraquinones in methanol + water. *J. Chem. Eng. Data* **1997**, *42*, 1212−1215.

(2) Ahmaed, I. T.; Soliman, E. S.; Boraei, A. A. A. The acidity constants of some pyrimidine bases in various water−organic solvent media. *Ann. Chim.* **2004**, 94, 847−856.

(3) El-Taher, M. A.; Gabr, A. A. Medium effect on acidity constants of some heterocyclic nitrogen azomethines. *Talanta* **1996**, *43*, 1511−1518.

(4) Beltran, J. L.; Sanli, N.; Fonrodona, G.; Barron, D.; Ozkan, G.; Barbosa, J. Spectrophotometric, potentiometric and chromatographic pK_a values of polyphenolic acids in water and acetonitrile−water media. *Anal. Chim. Acta* **2003**, *484*, 253−264.

(5) Tam, K. Y.; Hadley, M.; Patterson, W. Multiwavelength spectrophotometric determination of acid dissociation constants: Part IV. Water-insoluble pyridine derivatives. *Talanta* **1999**, *49*, 539−546.

(6) Qureshi, G. A.; Svehla, G.; Leonard, M. A. Electrochemical studies of strongly chelating anthraquinone derivatives. *Analyst* **1979**, *104*, 705−722.

(7) Thomson, R. H. *Naturally Occorring Quinones*; Academic Press: New York, 1971.

(8) Arcamone, F. Antitumor anthracyclines: recent developments. *Med. Res. Rev.* **1984**, *14*, 153−188.

(9) Lucker, M. *Secondary Metabolism in Microorganism, Plants and Animals*; Springer-Verlag: New York, 1984.

(10) Lowe, N. J.; Ashton, R. E.; Koudsi, H.; Verschoore, M.; Schaefer, H. Anthralin for psoriosis: short contact anthralin therapy compared with topical steroid and conventional anthralin. *J. Am. Acad. Dermatol.* **1984**, *10*, 69−75.

(11) Shamsipur, M.; Ghasemi, J.; Tamadon, F.; Sharghi, H. Spectrophotometric determination of acidity constants of some anthraquinones and anthrones in methanol−water mixtures. *Talanta* **1993**, *40*, 5, 697−699.

(12) Rived, F.; Canals, I.; Bosch, E.; Roses, M. Acidity of methanol−water. *Anal. Chim. Acta*, **2001**, *439*, 315−333.
(13) Kubista, M.; Sjoback, R.; Albinsson, B. Determination of equilibrium constants by chemometric analysis of spectroscopic data. *Anal. Chem.* **1993**, *65*, 994−998.
(14) Mwalupindi, A. G.; Rideau, A.; Agbria, R. A.; Warner, I. M. Influence of organized media on the absorption and fluorescence spectra of auramine-O dye. *Talanta* **1994**, *41*, 599−609.
(15) Vatena, G. C.; Bright, F. V. Thermodynamic study on the effects of β-cyclodextrin inclusion with anilinonaphthalenesulfonates. *Anal. Chem.* **1989**, *61*, 905−909.
(16) Liu, G.; Wu, T.; Zhao, J.; Hidaka, H.; Serpone, N. Photoassisted degradation of dye pollutants. 8. Irreversible degradation of alizarin red under visible light radiation in air-equilibrated aqueous TiO_2 dispersions. *Environ. Sci. Technol*. **1999**, *33* (12), 2081−2087.
(17) Iquierdo-Ridorsa, I.; Saurina, J.; Cassou, S. H.; Tauler, R. Second-order multivariate curve resolution applied to rank-deficient data obtained from acid−base spectrophotometric titrations of mixtures of nucleic bases *Chemom. Intell. Lab. Syst*. **1997**, *38*, 183−196.
(18) Saurina, J.; Cassou, S. H.; Tauler, R.; Izquierdo-Ridrosa, A. Spectrophotometric determination of pK_a values based on a pH gradient flow-injection system. *Anal. Chim. Acta*, **2000**, *408*, 135−143.
(19) Malinowski, E. R. *Factor Analysis in Chemistry*; John Wiley: New York, 1991.
(20) de Juan, A.; Maeder, M.; Martinez, M.; Tauler, R. Application of a novel resolution approach combining soft- and hard-modelling features to investigate temperature-dependent kinetic processes. *Anal. Chim. Acta* **2001**, *442*, 337−350.
(21) Ghasemi, J.; Niazi, A.; Kubista, M.; Elbergali, A. Spectrophotometric determination of acidity constants of 4-(2-pyridylazo)-resorcinol in binary methanol−water mixtures. *Anal. Chim. Acta* **2002**, *455*, 335−342.
(22) Ghasemi, J.; Ahmadi, Sh.; Kubista, M.; Forootan, A. Determination of acidity constants of 4-(2-pyridylazo)resorcinol in binary acetonitrile + water mixtures. *J. Chem. Eng. Data* **2003**, *48*, 1178−1184.
(23) Ghasemi, J.; Ghobadi, S.; Abbasi, B.; Kubista, M. Spectrophotometric determination of acidity constants of group B vitamins in different ionic strengths at 25 ± 0.1 °C. *J. Kor. Chem. Soc.* **2005**, *49*, 3, 1−9.
(24) Niazi, A.; Ghalie, M.; Yazdanipour, A.; Ghasemi, J. Spectrophotometric determination of acidity constants of Alizarine Reds in water, water-Brij-35, and water-SDS micellar media. *Spectrochim. Acta Part A* **2006**, *64*, 660−664.
(25) Niazi, A.; Yazdanipour, A.; Ghasemi, J.; Kubista, M. Multiwavelength spectrophotometric determination of acidity constants of morin in methanol−water mixtures. *Collect. Czech. Chem. Commun.* **2006**, *71*, 1−14.
(26) Kubista, M.; Sjoback, R.; Nygren, J. Quantitative spectral analysis of multicomponent equilibria. *Anal. Chim. Acta* **1995**, *302*, 121−125.
(27) Sacrminio, I.; Kubista, M. Analysis of correlated spectral data. *Anal. Chem.* **1993**, *65*, 409−416.
(28) Sjoback, R.; Nygren, J.; Kubista, M. Absorption and fluorescence properties of fluorescein. *Spectrochim. Acta Part A* **1995**, *51*, L7−L21.
(29) Nygren, J.; Andrade, J. M.; Kubista, M. Characterization of a single sample by combining thermodynamic and spectroscopic information in spectral analysis. *Anal. Chem.* **1996**, *68*, 1706−1710.
(30) Elbergali, A.; Nygren, J.; Kubista, M. An automated procedure to predict the number of components in spectroscopic data. *Anal. Chim. Acta* **1999**, *379*, 143−158.
(31) Kubista, M.; Nygren, J.; Elbergali, A.; Sjoback, R. Making reference samples redundant. *Crit. Rev. Anal. Chem.* **1999**, *29*, 1−18.
(32) Nygren, J.; Svanvik, N.; Kubista, M. Interaction between fluorescent dye thiazole orange and DNA. *Biopolymers* **1998**, *46*, 39−51.
(33) Svanvik, N.; Nygren, J.; Westman, G.; Kubista, M. Free probe fluorescence of light-up probes. *J. Am. Chem. Soc.* **2001**, *123*, 803−809.
(34) Ghasemi, J.; Niazi, A.; Westman, G.; Kubista, M. Thermodynamic characterization of the dimerization equilibrium of an asymmetric dye by spectral titration and chemometric analysis. *Talanta* **2004**, *62*, 835−841.
(35) Ghasemi, J.; Niazi, A.; Kubista, M. Thermodynamics study of the dimerization equilibria of rhodamine B and 6G in different ionic strengths by photometric titration and chemometrics method. *Spectrochim. Acta Part A* **2005**, *62*, 649−656.
(36) Niazi, A.; Yazdanipour, A.; Ghasemi, J.; Kubista, M. Spectrophotometric and thermodynamic study on the dimerization equilibrium of ionic dyes in water by chemometrics method. *Spectrochim. Acta Part A* (in press).
(37) http://www.multid.se.
(38) Rouhollahi, A.; Kiaie, F. M.; Ghasemi, J. Multiwavelength spectrophotometric determination of protolytic constants of 4-(2-pyridylazo) resorcinol (PAR) in binary DMF−water mixtures. *Talanta* **2005**, *66*, 653−658.
(39) Douheret, G. Comparison between glass electrode with hydroorganic and aqueous fillings. *Bull. Soc. Chim. Fr.* **1967**, 1412−1419.
(40) Douheret, G. Liquid junction potentials and effects of the medium glass-calomel electrode in such mixtures. *Bull. Soc. Chim. Fr.* **1968**, 3122−3131.
(41) Bishop, E. *Indicators*; Pergamon Press: Oxford, 1972.
(42) Kido, H.; Fernelius, W. C.; Hass, C. G. Studies on coordination compounds: formation constants of some metal salts of hydroxyquinones. *Anal. Chim. Acta* **1960**, *23*, 116−123.
(43) Coetzee, J. F.; Ritchie, C. D. *Solute−Solvent Interaction*; Marcel Dekker: New York, 1969.
(44) Azab, H. A.; Anwar, Z. M.; Sokar, M. Medium effect on the apparent dissociation constants of guanine, thymine, uracil, hypoxanthine, and cytosine in various hydroorganic media. *J. Chem. Eng. Data* **2004**, *49*, 256−263.
(45) Rageh, N. M. Acidity constants of some hydroxy azo pyrazolopyrimidines in mixed aqueous−organic solvents. *J. Chem. Eng. Data* **1998**, *43*, 373−379.

Received for review December 22, 2005. Accepted June 3, 2006.

JE050535D