CROATICA CHEMICA ACTA
CCACAA, ISSN 0011-1643, e-ISSN 1334-417X
Croat. Chem. Acta **82** (4) (2009) 753–759.
CCA-3369

Original Scientific Paper

Spectrophotometric Determination of the Dissociation Constants of Fluorescein in Micellar Media

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RECEIVED OCTOBER 4, 2006; REVISED APRIL 7, 2007; ACCEPTED DECEMBER 3, 2008

Abstract. The investigation of the dissociation equilibrium of fluorescein in aqueous micellar solution was determined spectrophotometrically at 25 °C and at ionic strength of 0.1 mol dm^{-3}. For this purpose, the effect of nonionic (TX100), cationic (CTAB) and anionic (SDS) surfactants on the absorption spectra of fluorescein at different pH values were studied. To evaluate the pH-absorbance data, a resolution 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 to an established factor analysis model. DATAN program was applied for determination of acidity constants. Results show that the pK_a values of fluorescein are influenced as the percentages of an anionic and a cationic surfactant such as SDS and CTAB, respectively, are added to the solution of this reagent. Also, neutral surfactant such as TX100 only affects pK_{a1}. Effects of surfactant on acidity constants and pure spectrum of each component are also discussed.

Keywords: fluorescein, acidity constants, TX100; SDS, CTAB, DATAN, spectrophotometric

INTRODUCTION

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 titration, 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] Various methods for the determination of dissociation constants, such as potentiometric titration, spectrophotometric determination, conductometry, and spectroscopic methods, have been reported. Among these, potentiometric titration[2] and spectrophotometric determination[3] are the most useful and widely used methods. For potentiometric titration, the dissociation constants of extremely acidic or basic compounds cannot be accurately determined because of their instability in an extreme pH range or because of the limitations of pH meters. Another essential requirement of this method is that the initial concentration of the samples must be accurately determined, it means, the samples must be pure and dry.

Aqueous micellar media are widely used in different areas of analytical chemistry and several reviews have been published related to them.[4–7] One important property of micelles is their ability to solubilize a wide variety of compounds which are insoluble or slightly soluble in water. The incorporation of a solute into micellar systems can lead to important changes in its molecular properties. Another important effect of micellar systems is that they can modify reaction rates and, to some extent, the nature of the products. Micelles can inhibit or accelerate reaction rates (by up to several orders of magnitude) and also shift the equilibria (acid-base). Surfactants usually affect spectral parameters: the intensity and shifts in the absorption bands can be increased, and shifts, in the absorption maxima of reagents are observed.[8,9] Micelles can affect the apparent pK_a values of the reagents due to a combination of elec-

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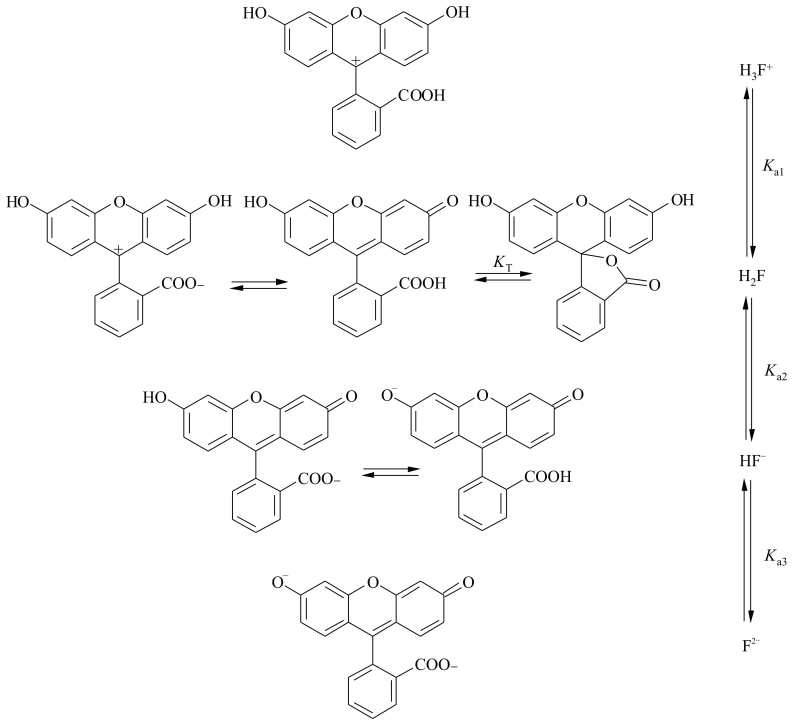

Figure 1. Protolytic equilibria of fluorescein.

trostatic and microenvironmental variations of the micelle.[10–12] Moreover, the acid-base equilibria involved in these systems are also influenced by surfactant.[13–17]

The fluorescein dye is probably the most common fluorescent probe today. Fluorescein is commercially available in many derivatives, such as isothiocyanate and fluorescein succinimidyl ester, that can be covalently attached to macromolecules and to amino acids. Fluorescein (Figure 1) in aqueous solution exists in cationic, neutral, anionic and dianionic forms making its absorption properties strongly pH dependent.[18] The protolytic constants relating the concentrations of protolytic forms have been difficult to determine, because their spectra overlap substantially and the different pK_a values are quite close. Using chemometrics methods, one can analyze whole spectra, thereby utilizing all spectrum information.[19,20] The approach is superior to any single-point measurement since several hundreds data points per spectrum can be treated simultaneously.[21] 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.[22,23] Data analysis was carried out by DATAN package that is developed by Kubista group,[24–30] the outputs of the fitting processes were acidity constants, spectral profiles of pure forms and other factor analysis data. The theory and application of physical constraints method were discussed in previous studies in several papers.[17,31–39]

In this study, we applied the DATAN program to determine the acidity constants of fluorescein in pure water, water-TX100, water-SDS and water-CTAB micellar media solutions at 25 °C and an ionic strength of 0.1 mol dm^{-3} spectrophotometrically. The effects of pol(oxyethylene)(9.5)*p*-(1,1,3,3-tetramethyl) (TX100) as nonionic surfactant, sodium n-dodecyl sulfate (SDS) as anionic surfactant and cetyltrimethylammonium bromide (CTAB) were studied on the dissociation constants and pure spectrum of fluorescein.

EXPERIMENTAL

Materials

Fluorescein, TX100, SDS, CTAB, hydrochloric acid, sodium hydroxide and potassium nitrate were analytical grade commercial products from Merck (Darmstad, Germany). These reagents were used without further purification. Standard stock solution of 9.0×10^{-4} mol dm^{-3} of fluorescein was prepared by dissolving appropriate amounts of fluorescein in water. The stock solutions of surfactants were prepared by dissolving weighted amounts of substances in appropriate amounts of water. All the solutions were prepared in deionized

water.

Instrumentation and software

A Perkin Elmer (Lambda 25) 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 300 and 600 nm. A HORIBA M-12 pH-meter furnished with a combined glass-saturated calomel electrode was calibrated with at least two buffer solutions at pH 3.00 and 9.00. All absorption spectra where digitized at five data points per nanometer in the wavelength 300−600 nm and transferred (in ASCII format) to an AMD 2000 XP (256 Mb RAM) computer for subsequent analysis by MATLAB software, version 6.5 (The MathWorks) or for processing by using DATAN package.

Spectrophotometric titrations

For the fluorescein (2.3×10^{-5} mol dm^{-3}) in pure water, water-TX100, water-SDS and water-CTAB mixtures titrations, absorption spectra were measured with a titration set-up consisting of a computer interfaced to a spectrophotometer. After each pH adjustment by hydrochloric acid and sodium hydroxide, solution was transferred into the cuvette and the absorption spectra were recorded. Ionic strength was maintained at 0.1 mol dm^{-3} by adding appropriate amounts of KNO_3. All measurements were carried out at the temperature (25 ± 0.5 °C).

RESULTS AND DISCUSSION

Figure 1, which describes the interconversions of protolytic species, has been discussed in previous reports,[40] implies low acidity for different groups. In previous reports value of acidity constants are obtained in pure water.[18] The protolytic constants relating the chemical activity of the cation, neutral form, anion and dianion are pK_{a1} = 2.08, pK_{a2} = 4.31 and pK_{a3} = 6.43. Song *et al.*[41] and Biswas *et al.*[42] investigated the absorption and fluorescence spectra of fluorescein in SDS, CTAB and TX100 micelles. Hadjianestis and Nikokavouras[43] have reported the absorption and fluorescence spectra of fluorescein in CTAB micelles. Also, Mchedlov-Petrossyan *et al.*[44,45] and Kibblewhite *et al.*[46] have reported of fluorescein ionization in different media.

The absorption spectra of fluorescein in pure water at various pH values at 300−550 nm intervals were recorded. In order to determine the influence of the nonionic surfactant (TX100), the anionic surfactant (SDS) and the cationic surfactant (CTAB) on acidity constants, a series of experiments were run at different TX100, SDS and CTAB concentrations. Figures 2−5 show the absorption spectra of fluorescein at different pH values in pure water, water-TX100, water-SDS and

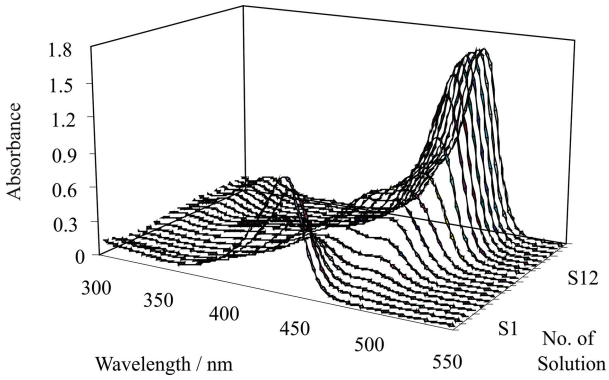

Figure 2. Absorption spectra of fluorescein in pure water at different pH values: (1) 1.00, (2) 1.57, (3) 2.12, (4) 2.56, (5) 3.05, (6) 3.58, (7) 4.01, (8) 4.52, (9) 5.02, (10) 5.57, (11) 6.02, (12) 6.50, (13) 7.05, (14) 7.56, (15) 8.01, (16) 8.57, (17) 9.05, (18) 10.09, (19) 11.04.

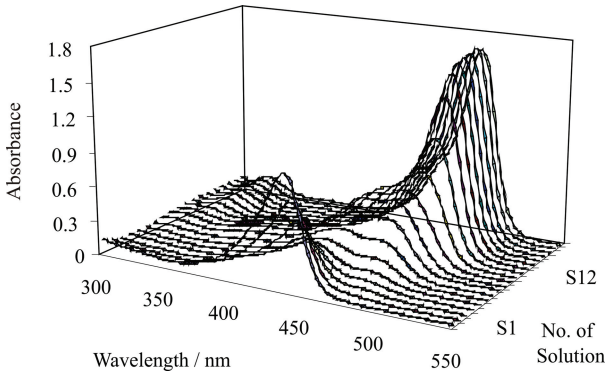

Figure 3. Absorption spectra of fluorescein in 0.025 *w*/*v* % TX100 to water at different pH values: (1) 1.03, (2) 1.51, (3) 2.09, (4) 2.50, (5) 3.01, (6) 3.55, (7) 4.05, (8) 4.55, (9) 5.04, (10) 5.51, (11) 6.04, (12) 6.49, (13) 7.10, (14) 7.54, (15) 8.00, (16) 8.55, (17) 9.08, (18) 10.10, (19) 11.16.

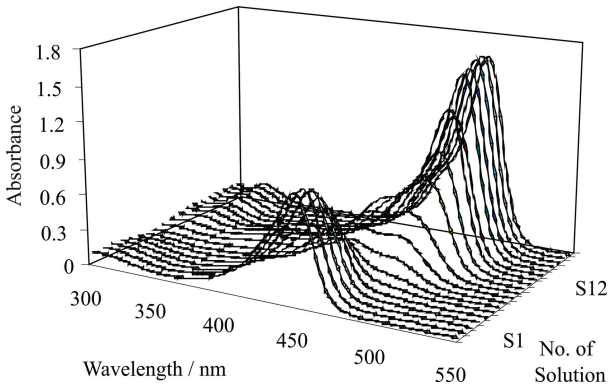

Figure 4. Absorption spectra of fluorescein in 0.05 *w*/*v* % SDS to water at different pH values: (1) 1.00, (2) 1.52, (3) 2.01, (4) 2.57, (5) 3.09, (6) 3.61, (7) 4.04, (8) 4.55, (9) 5.05, (10) 5.56, (11) 6.07, (12) 6.55, (13) 7.06, (14) 7.50, (15) 8.03, (16) 8.51, (17) 9.05, (18) 10.08, (19) 11.04.

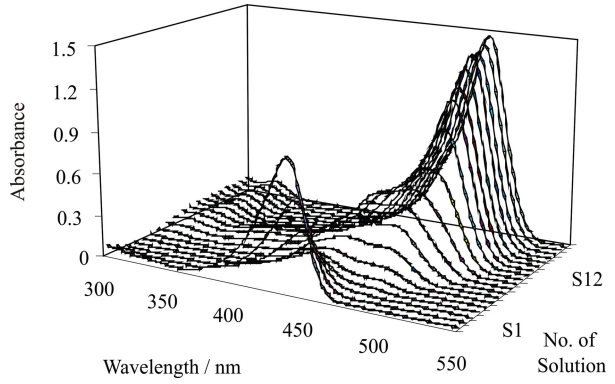

Figure 5. Absorption spectra of fluorescein in 0.05 *w*/*v* % CTAB to water at different pH values: (1) 1.02, (2) 1.53, (3) 2.03, (4) 2.52, (5) 3.02, (6) 3.51, (7) 4.03, (8) 4.51, (9) 5.01, (10) 5.51, (11) 6.03, (12) 6.52, (13) 7.02, (14) 7.51, (15) 8.01, (16) 8.53, (17) 9.03, (18) 10.04, (19) 11.03.

water-CTAB. The shape of absorption spectra of fluorescein in TX100 micelles is very close to that in aqueous solution. But, the red shifts in absorption spectra of fluorescein in cationic (CTAB) and anionic (SDS) micelles are observed. Singular value decomposition (SVD) analysis performed on all absorption data matrices obtained at various pH values for fluorescein gives the number of components that best represent the system. SVD is the preferred algorithm because it has the most stability under the widest range of application.[21] The SVD algorithm decomposes an arbitrary data matrix, **A**, with more rows than columns, into three matrices, $\mathbf{A}=\mathbf{USV}^{T}$. The column of the matrix **U** (which has the same dimensions as **A**), constitutes the so-called basis spectra of **A**. SVD has the very useful property of compressing the information contained in **A** into the first few columns of **U**, such columns are mutually orthogonal (linearly independent) and their importance decreases as rapidly as possible after the first column. The importance of each column of **U** in representing **A** is given by the squares of nonnegative diagonal values (singular values) of $\mathbf{S}.\mathbf{V}^{T}$ (where T means the transpose) describes the composition of **A** in terms of basis spectra. **V** and **S** have dimensions equal to the number of columns of **A**. Four significant factors are also supported by the statistical indicators of Elbergali *et al.*[30] These factors could be attributed to the three dissociation equilibria of a triprotic acid such as fluorescein. The pK_a values of fluorescein were investigated in pure water, different water-TX100, water-SDS and water-CTAB mixtures spectrophotometrically at 25 °C and an ionic strength of 0.1 mol dm^{-3}.

Acidity constants of fluorescein in several mixtures were evaluated using the DATAN program with the corresponding spectral absorption-pH data. From inspection of the experimental spectra, it is hard to guess even the number of protolytic species involved.

Table 1. Acidity constants of fluorescein in pure water and at different *w*/*v* percentage of TX100 at 25°C and constant ionic strength (0.1 mol dm^{-3} KNO_3)

TX100 / %(*w*/*v*)	pK_{a1}	pK_{a2}	pK_{a3}
0.00	1.93 ± 0.09	4.15 ± 0.14	6.44 ± 0.20
0.001	1.82 ± 0.08	4.15 ± 0.15	6.46 ± 0.20
0.005	1.74 ± 0.09	4.18 ± 0.16	6.45 ± 0.21
0.01	1.65 ± 0.09	4.20 ± 0.16	6.45 ± 0.20
0.025	1.31 ± 0.10	4.21 ± 0.17	6.48 ± 0.21

Table 2. Acidity constants of fluorescein in pure water and at different *w*/*v* percentage of SDS at 25°C and constant ionic strength (0.1 mol dm^{-3} KNO_3)

SDS / % (*w*/*v*)	pK_{a1}	pK_{a2}	pK_{a3}
0.00	1.93 ± 0.09	4.15 ± 0.14	6.44 ± 0.20
0.001	2.03 ± 0.11	4.28 ± 0.14	6.85 ± 0.23
0.005	2.13 ± 0.11	4.51 ± 0.15	7.06 ± 0.25
0.01	2.30 ± 0.12	4.85 ± 0.16	7.33 ± 0.24
0.025	3.11 ± 0.15	5.05 ± 0.17	7.70 ± 0.24
0.05	3.27 ± 0.15	5.29 ± 0.17	7.81 ± 0.26

Table 3. Acidity constants of fluorescein in pure water and at different *w*/*v* percentage of CTAB at 25°C and constant ionic strength (0.1 mol dm^{-3} KNO_3)

CTAB / %(*w*/*v*)	pK_{a1}	pK_{a2}	pK_{a3}
0.00	1.93 ± 0.09	4.15 ± 0.14	6.44 ± 0.20
0.001	1.93 ± 0.10	4.16 ± 0.14	6.48 ± 0.21
0.005	1.92 ± 0.10	4.17 ± 0.15	6.50 ± 0.21
0.01	1.91 ± 0.11	4.17 ± 0.15	6.52 ± 0.23
0.025	1.41 ± 0.11	4.33 ± 0.16	6.63 ± 0.24
0.05	0.94 ± 0.10	4.44 ± 0.16	6.70 ± 0.24

The most four calculated significant projection vectors with clear spectral features (as compared to noise) evidence the presence of four spectroscopically distinguishable components. Their shapes, however, are clearly unphysical and cannot be directly related to the spectral response of the four protolytic forms. The outputs of the program are pK_a values, their standard deviation, the number of principal components, projection vectors (loadings) and the pure spectrum of each assumed species.

The results obtained by the application of the DATAN program are summarized in Tables 1−3. These pK_a values clearly show the different behavior of fluorescein in these different charge-type micellar systems, pK_a values are also shown in Figure 6. As can be seen from Figure 6, it can be concluded that the surfactant TX100, only causes a decrease in pK_{a1}. According to the increases of acidity constant of K_{a1}, it can be obtained that a neutral surfactant such as TX100, has larger interaction with H_2F. And ionic species of fluorescein does not

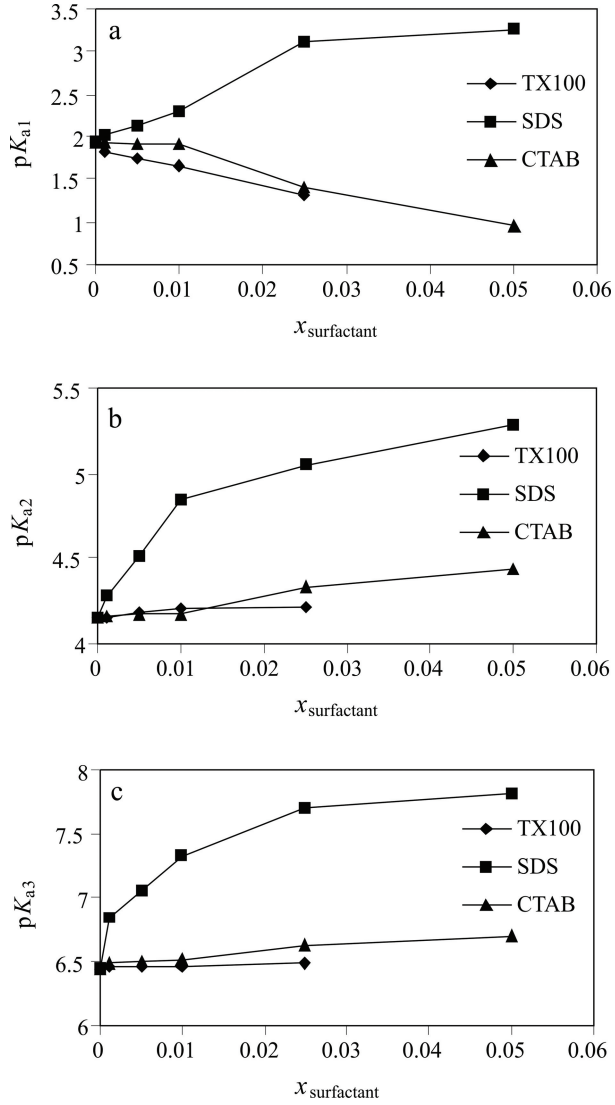

Figure 6. Variation of acidity constants values of fluorescein with percentages of different surfactant. (a) pK_{a1}, (b) pK_{a2} and (c) pK_{a3}.

have considerable interaction with TX100. According to the pH changes in the region of H_2F production, absorption is observed. It can be thought that the species H_2F which has the lactone form is not formed in solution and tautomeric reaction does not proceed. When the effect of the SDS is studied (Table 2), it is observed that all pK_a's are increased (decrease of acidity constants), and it shows that anionic surfactant such as SDS, interacts with cationic species (H_3F^+) or species which are less anionic. And finally, effect of CTAB (Table 3) causes a decrease in pK_{a1} and increases pK_{a2} and pK_{a3}, which can be concluded that CTAB has more interaction with H_2F.

The pK_a values depend on absorption spectrum variation at different pH in all micelles systems. One of the very important outputs of the DATAN program is the calculated spectrum of different forms of fluorescein in each micellar media. Sample spectrum of the calculated spectra of all species in pure water, 0.025 *w*/*v* % TX100, 0.05 *w*/*v* % SDS and 0.05 *w*/*v* % CTAB to water are shown in Figure 7. It is interesting to note that the nature of the surfactant has a fundamental effect on each pure spectrum. As it is clear from Figure 7, this effect is apparently observed in different species of fluorescein. The surfactant effect on this spectrum is very interesting. Many papers and reviews have discussed the effect of micelle on the apparent pK_a values of the acids.[4–17] In the present work we observed the changes of spectrum in TX100, SDS and CTAB micelles systems and then we calculated the pK_a values of this reagent in these media. As it is clear from Figure 7, when the TX100 surfactant is used, it causes higher intensity in spectra of H_3F^+. The pK_{a1} is decreased and pK_{a2} and pK_{a3} values are increased slightly in different percentages of TX100. By using SDS surfactant, the most changes are observed in spectra of H_2F and HF^-. The acidity constant values are increased with the increase of SDS percentages. In the case of CTAB surfactant, changes in spectra cause: pK_{a1} is unchanged up to 0.01 % (*w*/*v*) and then decreases, pK_{a2} increases slightly till 0.01 % (*w*/*v*) and then increases more and finally in the case of pK_{a3} there is an increase. Absorption maxima positions for different species of fluorescein in different micellar media are shown in Table 4. It is interesting to note that the nature of the micelle composition has a fundamental effect on pure spectrum intensities and not on maxima positions of spectrum. According to the results of Table 4, the most changes of maxima position of spectrum is related to SDS.

Table 4. Spectral characteristics of different species in pure water and micellar solutions at 25°C and constant ionic strength (0.1 mol dm^{-3} KNO_3)

Species of fluorescein	λ_{max} / nm			
	Water	TX100 0.025 % (*w*/*v*)	SDS 0.05 % (*w*/*v*)	CTAB 0.05 % (*w*/*v*)
H_3F^+	440	441	449	441
H_2F	438	439	445	437
HF^-	478	479	482	477
F^{2-}	493	492	493	493

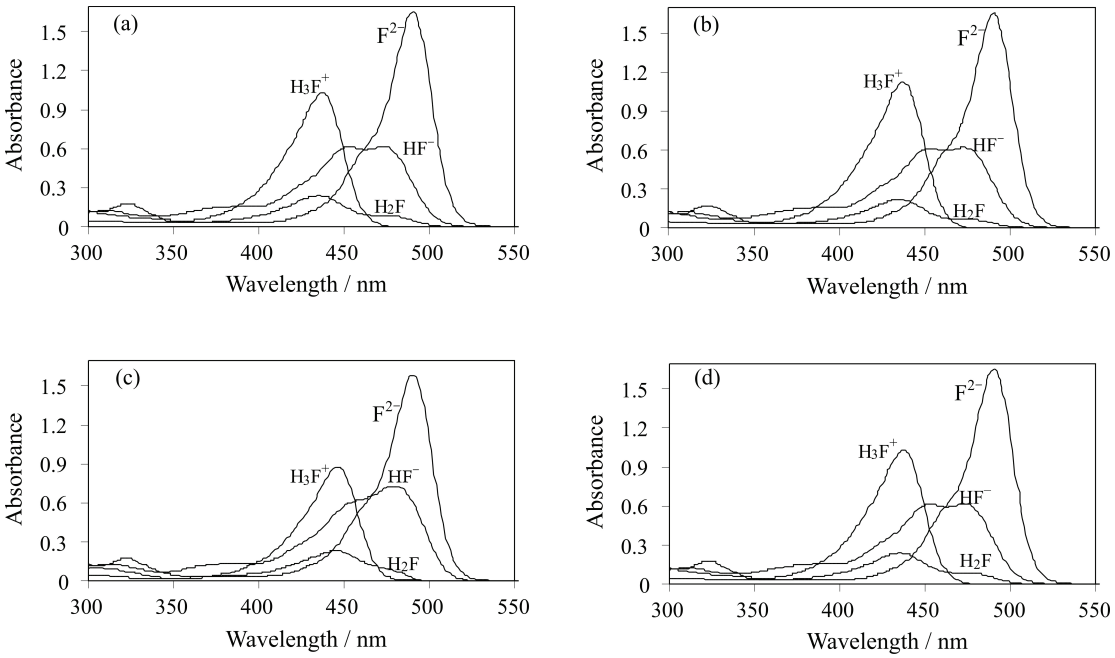

Figure 7. The pure spectra of different form of fluorescein in (a) Pure water, (b) 0.025 % (*w*/*v*) TX100 to water, (c) 0.05 % (*w*/*v*) SDS to water and (d) 0.05 % (*w*/*v*) CTAB to water.

The difference between the pK_a calculated by presented method, and the pK_a exist in literature can be because of, experimental errors and the oldness of last methods, in comparison with chemometrics methods in which, all of the spectrum is used for analysis. So the obtained acidity constants by DATAN are more reliable and precise than previous methods. These changes are due to the hydrophobic and electrostatic interactions of reactants with micellar aggregates.[4–17,40–48]

CONCLUSION

In this work, we distinguish the behavior of acidity constants of fluorescein in pure water, water-TX100, water-SDS and water-CTAB micellar media systems at 25 °C and an ionic strength of 0.1 mol dm^{-3} that are studied by multiwavelength spectrophotometric method. Results show that the pK_a values of fluorescein are influenced as the percentages of an anionic and a cationic surfactant such as SDS and CTAB, respectively, added to the solution of this reagent. Also, neutral surfactant such as TX100 only affects pK_{a1}. DATAN is a useful tool for resolution of the different species present in equilibria systems. By using this method and without any prior knowledge about the system, we can obtain the acidity constants and pure spectra from the experimental data. In conclusion, interaction with micellar aggregates induces significant pK_a shifts which can be rationalized in terms of partitioning of species and electrostatic contribution.

Acknowledgements. The authors are gratefully acknowledging the support of this work by the Islamic Azad University, Arak Branch research council.

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SAŽETAK

Spektrofotometrijsko određivanje konstanti disocijacije flouresceina u micelarnom mediju

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Ravnotežna disocijacija fluoresceina u vodenoj micelarnoj otopini je istraživana spektrofotometrijski pri 25 °C i pri ionskoj jakosti 0.1 mol dm^{-3}. Za to je istraživan učinak neionskih (TX100), kationskih (CTAB) i anionskih (SDS) surfaktanata na apsorpcijske spektre fluoresceina pri različitim pH vrijednostima. Da se procijene podaci pH-absorbancija, upotrijebljeno je razlučivanje temeljeno na kombinaciji mekog i tvrdog modeliranja (engl. *soft- and hard-modeling*). Konstante kiselosti svih odgovarajućih ravnoteža su određene korištenjem ugađanja cijelih spektara na uspostavljeni faktorski model. DATAN program je upotrijebljen za određivanje konstanti kiselosti. Rezultati pokazuju da na pK_a vrijednosti fluoresceina utječu čak i postoci dodanih anionskih i kationskih surfaktanata poput SDS-a i CTAB-a. Neutralni surfaktant, poput TX100 utječe samo na pK_{a1}. Također su istraženi utjecaji surfaktanata na konstante kiselosti i čistog spektra svake komponente.