*J. Braz. Chem. Soc.*, Vol. 21, No. 10, 1952-1960, 2010.
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0103 - 5053 $6.00+0.00

Article

# Determination of p$K_a$ Values of Some Sulfonamides by LC and LC-PDA Methods in Acetonitrile-Water Binary Mixtures

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As constantes de dissociação de sete sulfonamidas antibióticas, sulfadiazina, sulfatiazol, sulfamerazina, sulfametazina, sulfamonometoxina, sulfadoxina e sulfametoxazol, foram determinadas em diferentes misturas binárias acetonitrila-água (15, 30, 40, 50% (v/v)) usando cromatografia líquida de fase reversa. Um método usando espectros de absorbância no máximo dos picos cromatográficos previamente obtidos foi também aplicado para determinar os valores de p$K_a$. Este método pode ser aplicado a dados oriundos de equipamentos LC-UV (com detecção por arranjo de diodos, PAD) retendo todas as vantagens dos métodos LC e espectrofotométricos. Correlações lineares foram observadas quando os valores calculados de p$K_a$ das sulfonamidas em diferentes misturas de solventes foram comparados com a fração molar da acetonitrila.

The dissociation constants of seven sulfonamide antibiotics, sulfadiazine, sulfathiazole, sulfamerazine, sulfamethazine, sulfamonomethoxine, sulfadoxine and sulfamethoxazole, have been determined in different acetonitrile-water binary mixtures (15, 30, 40, 50% v/v) by means of reversed-phase liquid chromatographic data. Also, a method based on the absorbance spectra at the maximum of chromatographic peaks previously obtained has been applied to determine the p$K_a$ values. This method can be applied to data obtained from LC-UV (photodiode array detection (PDA)) instruments and retains all the advantages of LC and spectrophotometric methods. Linear relationships were observed when the calculated p$K_a$ values of sulfonamides in different solvent mixtures were plotted against the acetonitrile molar fraction.

**Keywords:** sulfonamides, liquid chromatography, PDA, p$K_a$ values

## Introduction

Sulfonamides (SAs, substituted amides of sulfanilic acid at the N-1 position) are anti-bacterial and anti-infective compounds used preferably in farm animals for the treatment of a variety of bacterial infections. In food-producing animals SAs are used not only for the treatment of several diseases but also for prophylactic purposes and/or for promotion of growth. A major concern with the use of these compounds is that residues may be present in animal food products and may pose a health threat to consumers.[1-5]

Over the years, several separation methods with several detectors have been developed and used for the determination of SAs in various samples. These procedures have generally employed reversed-phase high performance liquid chromatography (RP-HPLC) with either UV,[6,7] fluorimetric[8,9] or electrochemical[10] detection techniques. The used mobile phase was usually composed of a relatively high proportion of an organic solvent (*e.g.* acetonitrile (MeCN) or methanol) and an acidic aqueous buffer in order to elute SAs from the column in a few minutes. The low pH of the buffer was intended to keep SAs in its neutral form given that this compound may be ionized at high pHs.

The dissociation constant (p$K_a$) of a drug molecule is a key parameter in absorption, distribution, metabolism,

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excretion and toxicity researches because it governs solubility, absorption, distribution, and elimination of substances.[11] Also, the p$K_a$ values constitute important data for thorough understanding of certain chemical phenomena such as biological uptake, and the binding of these molecules to environmental matrices and forming chelates with metallic cations. The drugs' p$K_a$ data are applied to estimate the major species of pharmaceuticals present in the environment (usually in neutral pH range) and dosage-form development.

The protonation of the analyte in HPLC is regarded as a secondary chemical equilibrium, the first being the distribution equilibrium of the solute between the mobile and stationary phase. The secondary equilibrium is controlled by both the pH of the mobile phase and the ratio of the studied organic modifier. The basic theory of these equilibria has been worked out in the 1970s and 1980s.[12,13] The analyte retention in reversed-phase chromatography represents the molecular fraction weighted average of the retention of the dissociated and undissociated forms.

In the study of dissociation equilibria by reversed phase liquid chromatography, the compounds under study can be considered to be typical amphoteric compounds throughout the working pH range. The overall dissociation process can be shown as:

$$H_2B^+ \underset{}{\overset{K_{a1}}{\rightleftharpoons}} HB + H^+ \overset{K_{a2}}{\rightleftharpoons} B^- + 2H^+ \quad (1)$$

The molecular form HB, ionizes in a cationic form, $H_2B^+$, as the pH decreases, and ionizes in an anionic form, $B^-$, as the pH increases. Figure 1 shows the two-step dissociation pathway of sulfonamides. $K_{a1}$ and $K_{a2}$ are the dissociation constants of the aromatic amine and sulfonic groups, respectively.

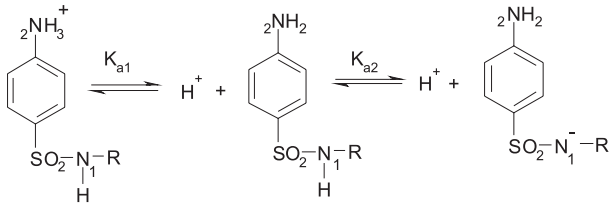



**Figure 1.** Protolytic equilibria of sulfonamides.

The expression of the observed retention factors ($k = t_R - t_o / t_o$, where $t_R$ is the analyte retention time and $t_o$ is the void volume) can be given by

$$k_{obs} = \frac{k_0 + k_{-1}\frac{K_{a1}}{[H^+]} + k_1\frac{[H^+]}{K_{a2}}}{1 + \frac{K_{a1}}{[H^+]} + \frac{[H^+]}{K_{a2}}} \quad (2)$$

where $k_0$, $k_{-1}$ and $k_1$ are the retention factors of the neutral, the anionic, and the cationic forms of the ampholyte and $K_{a1}$ and $K_{a2}$ are the corresponding acid dissociation constants, respectively.

Debye-Hückel equation was used to compute the activity coefficients:

$$-\log\gamma = \frac{Az^2\sqrt{I}}{1 + a_0B\sqrt{I}} \quad (3)$$

where $\gamma$ is the activity coefficient of the involved species, z is the ion charge of the ion and I the ionic strength of the solution. A and $a_0$B values for MeCN-water mixtures are taken from Barbosa *et al.*[14-16] The variation of the activity coefficients is changed in the range of 0.994 to 0.881 in the different mobile phases and the pH values.

There are lots of p$K_a$ determination techniques for compounds of pharmaceutical or biological interest such as potentiometric titrations,[17,18] UV-Vis,[19,20] LC[21,22] and software computational prediction.[23,24] Among these techniques, liquid chromatography is used as a powerful method for determination of dissociation constants because it requires only small quantity of compounds, studied samples do not need to be pure and poor water solubility is not a serious drawback. This method does not requires measuring solute or titrant concentrations, just only retention times. Also, calculation is straightforward and independent of solute purity. Also a method based on the absorbance spectra at the maximum of chromatographic peak obtained along with photo diode array (PDA) detection has been applied. This method can be applied to data obtained from LC-PDA instruments and retains all the advantages of LC and spectrophotometric methods, such as the possibility of working with impure samples.

SAs contain ionogenic functions such as aromatic amine ($H_3N^{(+)}$-$C_6H_4$-$SO_2$-NHR) (p$K_{a1}$) and sulfonic ($H_2N$-$C_6H_4$-$SO_2$-$N^{(-)}$-R) (p$K_{a2}$) groups, respectively. Their retention on column depends on the percentage of ionized and non-ionized species of each compound. Thus, knowledge of the acid-base dissociation constants of SAs in MeCN-water mixtures, which are usually used as the mobile phase, can help to improve the analytical method and can lead to a better understanding of the chromatographic behavior of these compounds. Although there are several publications related to the dissociation constants of SAs in water,[18,25–27] but there are no data on the p$K_a$ values of SAs in MeCN-water binary mixtures, which is the most widely used mobile phase for the separation of these compounds using liquid chromatography.

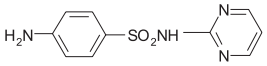
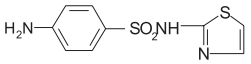
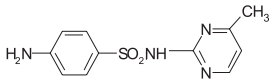
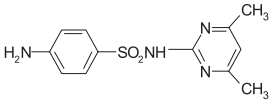
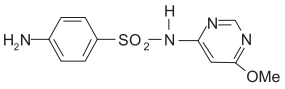
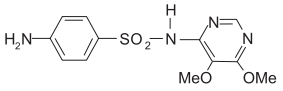
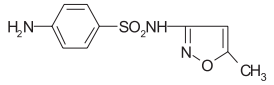
In this work, the p$K_a$ values of the seven SAs, sulfadiazine, sulfathiazole, sulfamerazine, sulfamethazine, sulfamonomethoxine, sulfadoxine and sulfamethoxazole, were determined using chromatographic data for mixture with 15-50% (v/v) MeCN.

## Experimental

### *Chemicals and reagents*

The standard SAs studied are shown in Table 1. They were purchased from Sigma and used without further purification. Water, with conductivity lower than 0.05 mS cm$^{-1}$ was obtained with a Milli Q water purification system (Milli Pore Corp.). MeCN was of HPLC grade and used as the organic component of the mobile phase.

**Table 1.** Chemical structures of studied sulfonamides

| Compounds | Chemical structures |
|---|---|
| Sulfadiazine, (4-Amino-*N*-(2-pyrimidin-2-yl) benzenesulfonamide) |  $C_{10}H_{10}N_4O_2S$ |
| Sulfathiazole, (4-Amino-*N*-(1,3-thiazol-2-yl) benzenesulfonamide) |  $C_9H_9N_3O_2S_2$ |
| Sulfamerazine, (4-Amino-N-(4-methylpyrimidin-2-yl) benzenesulfonamide) |  $C_{11}H_{12}N_4O_2S$ |
| Sulfamethazine, (Sulfadimidine), (4-Amino-*N*-(4,6-dimethylpyrimidin-2-yl) benzenesulfonamide) |  $C_{12}H_{14}N_4O_2S$ |
| Sulfamonomethoxine, (4-Amino-*N*-(6-methoxy-4-pirimidinil) benzenesulfonamide) |  $C_{11}H_{12}N_4O_3S$ |
| Sulfadoxine, (4-Amino-*N*-(5,6-dimethoxy-4-pyrimidinyl) benzenesulfonamide) |  $C_{12}H_{14}N_4O_4S$ |
| Sulfamethoxazole, (4-Amino-*N*-(5-methyl-3-isoxazolyl) benzenesulfonamide) |  $C_{10}H_{11}N_3O_3S$ |

Stock standard solutions of SAs were freshly prepared in water at concentrations of approximately 200 mg L$^{-1}$ and stored in amber bottles in refrigerator (4 °C). Working solutions were diluted with corresponding mobile phase to 10 mg L$^{-1}$. These solutions were passed through a 0.45 mm nylon filter membrane (MSI) before injections.

The hold-up time, $t_o$, was measured for every mobile phase composition by injection of 0.01% (m/v) potassium bromide solution (Merck).

### *Apparatus*

A chromatographic system consisted of Shimadzu Model LC 10 ADVP pump with an auto injector (SIL 10 AD VP) and diode array detector system (SPDM 10 A DAD) was used for studies. This equipment has column oven (CTO 10 AVP) and degasser system (DGU 14 A). A Phenomonex Prodigy ODS-3 100 A$^0$ (250×4.60 mm i.d. × 5 mm) end-capped, monomeric column with 15.5% carbon load was used at 25 °C.

The electromotive force (e.m.f) measurements used to evaluate the pH of the mobile phase were performed using Metleer-Toledo MA 235 pH/ion analyser with a Hanna HI 1332 combination pH electrode. The calibration solutions were thermostated externally at 25 ± 0.1 °C with a cooler system water bath (HETO CBN 8-30 and temperature control unit HETO HMT 200) when adjusting the pH. The electrode was stabilized in the appropriate MeCN-water mixture prior to e.m.f. measurements. pH measurements of the mobile phases were performed in triplicate to ensure stability and reproducibility of the potentiometric system.

The chromatographic retention of ionizable compounds is strongly dependent on the pH of the mobile phase. Thus an accurate measurement and control of mobile phase pH is required, in many instances, for efficient separations of ionizable compounds by HPLC.[28,29]

Several procedures are used to measure the mobile phase pH. The most common procedure is to measure the pH of the aqueous buffer before mixing it with the organic modifier, $^{w}_{w}$pH. A more rigorous procedure, recommended by the IUPAC, is to measure the pH of the mobile phase after mixing the aqueous buffer and the organic modifier. In this instance, the electrode system used to measure pH can be calibrated either with aqueous buffers, $^{s}_{w}$pH, or with buffers prepared in the same solvent composition used as mobile phase, $^{s}_{s}$pH. This requires knowledge of the pH value of reference buffers prepared in different aqueous–organic solvent mixtures.[30]

As pH values have been previously determined in MeCN-water mixtures for the primary standard series of substances proposed by NIST, in accordance with the IUPAC rules, $^{s}_{s}$pH values in MeCN-water mixtures can be measured.[31-34] In this study we used potassium hydrogen phthalate as primary standard buffer reference solutions in the MeCN-water mixtures studied. The molar activity coefficients, γ, were calculated using equation 3.

*Procedure*

*Liquid chromatographic method*

Throughout this study, the compounds were injected using isocratic system and the mobile phases assayed were MeCN-water at different compositions of acetonitrile (15:85, 30:70 and 40:60, 50:50 v/v) with trifluoroacetic acid, *o*-phosphoric acid and diethylmalonic acid. These buffers were preferred because of their appropriate p$K_a$ values. The pH of the mobile phases used was adjusted between 1.7 and 9.0 after addition of desired amount of sodium hydroxide. The mobile phase was prepared daily, filtered, sonicated before use and delivered at a flow rate of 1.0 mL $min^{-1}$ and the effluent was monitored at 270 nm. The mobile phase mixtures were filtered through a 0.45 μm pore nylon membrane filters (Millipore, Bedford, MA). A total of 10 μL of each solution was injected and chromatograms were recorded.

In general, for each MeCN content, the chromatographic retention was studied from acidic region to basic pH. The column was pre-conditioned during at least 1 h at low flow rate (0.5 mL $min^{-1}$) with mobile phase at the corresponding pH before the first injection. Retention factors were calculated as $k = (t_R - t_0)/t_0$, where $t_0$ indicates the hold-up time.

The p$K_a$ values were determined by performing a non-linear fit using the NLREG programme.[35] This is a general purpose program, where the function to be minimized and the parameters to be estimated can be defined by means of the built-in program editor. Data pairs of *k*-pH, ionic strength and the guessed p$K_a$ values and the retention factors of the fully protonated and deprotonated species are imported to the program. The ionic strength was determined from the amount of sodium hydroxide added to obtain the desired pH of the mobile phase, and from the dissociation constant of the buffer in the more acidic solutions. NLREG refines these parameters according to equation 4 to give a minimum in the sum of square residuals ($U_m$) in order to obtain the dissociation constants of the SAs studied.

$$U_m = \sum_{j=1}^{n_s} (k_{i,\exp} - k_{i,calc})^2 \quad (4)$$

where $n_s$ indicates the number of solutions, $k_{i,\exp}$ the experimental value of the retention factor for solution, *i*, and $k_{i,calc}$ the calculated value. The calculated retention factors are obtained from equation 2.

*LC-PDA method*

While using the spectral data, the method based on the absorbance spectra at the maximum of chromatographic peak, obtained with PDA has been applied to calculate the p$K_a$ values. This method allows comparing the values of dissociation constants obtained from chromatographic retention of SAs at different pH values and those obtained from absorbance spectra at the maximum of the chromatographic peak. Absorbance spectra were recorded between 190 and 500 nm and then processed by modified STAR (Stability Constants by Absorbance Readings) program.[36,38] The STAR program provides several statistical parameters to test the reliability of the regression process and the results obtained. The program gives the sum of the squared residuals, standard deviation of the residuals and the Hamilton R-factor (in %). Other valuable parameters for the examination of the distribution of the residuals are the Skewness and Kurtosis tests.

By checking the statistical parameters, we decide that the obtained results are good enough to be published or the experimental points could be repeated. In all cases, STAR program gives satisfactory statistical results in order to obtain the p$K_a$ values of studied compounds.

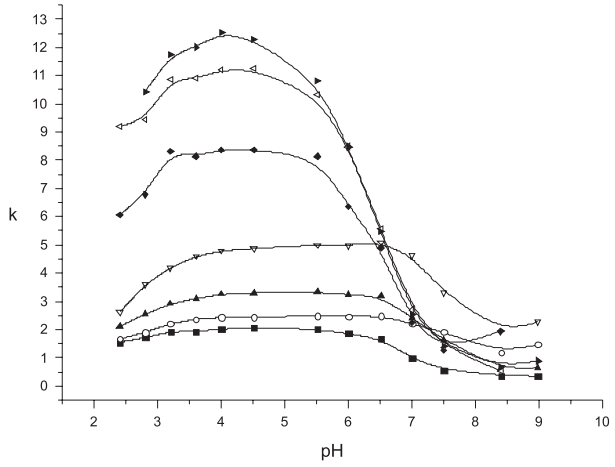
## Results and Discussion

SAs are ordinary ampholyte compounds so that their ionization can be described as a two-step protolysis (Figure 1). In ordinary ampholytes, when the difference ($\Delta pK_a$) between acidic p$K_a$ and basic p$K_b$ is greater than 3, only one kind of group (acid or basic) can be ionized to any extent at a time. However, when $\Delta pK_a$ is lower than 3, the ionization of the other group will no longer be negligible, causing the existence of a small proportion of the zwitterionic species. When pH is about equal to average p$K_a$, the neutral form is the dominant species in ampholytes. The hydrophobic nature of the neutral species is naturally greater than those of the associated ions.[39,40]

A sulfonamide contains two important functional groups in the pharmaceutically relevant pH range of 4 to 9 as shown in Figure 1: one acidic amide moiety ($N^1$) and one basic amine moiety ($N^2$). The amine nitrogen atom ($-NH_2$) is able to gain a proton, while the amide nitrogen atom (-NH-) is able to release a proton under specific pH conditions. Thus, the first dissociation equilibrium refers to the dissociation of amine moiety (p$K_1$) and the second equilibrium to the dissociation of amide moiety (p$K_2$).

In order to obtain the p$K_a$ values of SAs using LC methodology, data pairs of pH and retention factors, *k*, and the ionic strength over pH range of 1.7-9.0, were used. The aqueous-organic mixtures used as mobile phase were MeCN-water mixtures with percentages of 15, 30, 40 and 50% v/v MeCN. The *k* values were determined from three separate injections at every mobile phase

composition at each pH considered. In Figure 2, data pairs of *k*/pH for studied sulfonamides in 15% (v/v) MeCN are shown, together with the corresponding experimental and calculated retention factors from equation 2.




| Compounds | $k_{exp}$ | $k_{calc}$ |
|---|---|---|
| Sulfadiazine (■) | $k_{-1}$ : 1.55 (0.02)* | $k_{-1}$ : 1.19 (0.26) |
| | $k_0$ : 2.01 (0.01) | $k_0$ : 2.11 (0.05) |
| | $k_1$ : 0.35 (0.01) | $k_1$ : 0.30 (0.05) |
| Sulfathiazole (○) | $k_{-1}$ : 1.66 (0.01) | $k_{-1}$ : 1.21 (0.11) |
| | $k_0$ : 2.48 (0.01) | $k_0$ : 2.48 (0.01) |
| | $k_1$ : 1.18 (0.01) | $k_1$ : 1.06 (0.07) |
| Sulfamerazine (▲) | $k_{-1}$ : 2.12 (0.01) | $k_{-1}$ : 1.47 (0.10) |
| | $k_0$ : 3.32 (0.01) | $k_0$ : 3.32 (0.01) |
| | $k_1$ : 0.58 (0.01) | $k_1$ : 0.48 (0.10) |
| Sulfamethazine (▽) | $k_{-1}$ : 2.62 (0.02) | $k_{-1}$ : 1.04 (0.39) |
| | $k_0$ : 5.09 (0.01) | $k_0$ : 4.98 (0.03) |
| | $k_1$: 1.91 (0.01) | $k_1$: 1.85 (0.26) |
| Sulfamonomethoxine (♦) | $k_{-1}$ : 6.06 (0.01) | $k_{-1}$ : 6.05 (0.10) |
| | $k_0$ : 8.37 (0.01) | $k_0$ : 8.48 (0.20) |
| | $k_1$: 1.22 (0.02) | $k_1$: 0.33 (0.42) |
| Sulfadoxine (◁) | $k_{-1}$ : 9.19 (0.02) | $k_{-1}$ : 8.63 (0.37) |
| | $k_0$: 11.24 (0.03) | $k_0$: 11.34 (0.06) |
| | $k_1$: 0.54 (0.01) | $k_1$: 0.40 (0.07) |
| Sulfamethaxazole (▶) | $k_{-1}$ :10.42(0.02) | $k_{-1}$ : - |
| | $k_0$:12.52 (0.03) | $k_0$: 12.48 (0.09) |
| | $k_1$: 0.86 (0.01) | $k_1$: 0.62 (0.10) |

*The values between parentheses are the standard deviations.

**Figure 2.** Experimental and calculated retention factors, *k*, of neutral, anionic, and cationic forms of studied sulfonamides using equation 2 by NLREG program and plot of *k* vs. the pH of the mobile phase for 15% (v/v) MeCN.

A method based on the absorbance spectra at the maximum of chromatographic peak, obtained with PDA has been applied to obtain the p$K_a$ values. This method allows comparing the values of dissociation constants obtained from chromatographic retention of series of SAs at different pH values and those obtained from absorbance spectra at the maximum of the chromatographic peak. It is possible to constitute a valuable means of obtaining better precision.

As an example of the application of LC-UV (PDA) method, Figure 3 shows the variation of the absorbance spectra for sulfamethaxazole in the maximum of the chromatographic peaks over the pH range from 1.7 to 9.0 when working in 40% (v/v) MeCN media. This method retains the advantages of both methods (UV-Vis and LC). The two methods proposed can be used simultaneously without an increase in the experimental time and allow confirmation of the results obtained.

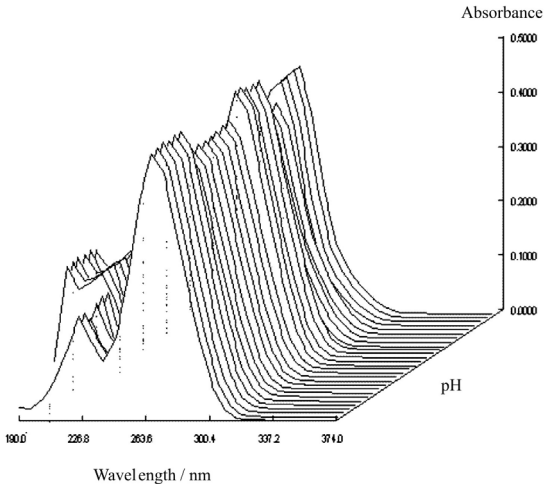



**Figure 3.** Wavelength (nm)-absorbance spectra for sulfamethoxazole as a function of pH at the maximum of LC peaks in 40% (v/v) MeCN-water mixtures.

The dissociation constant values determined for the equilibria involved for studied SAs in 15, 30, 40 and 50% v/v MeCN-water mixtures at 25.0 ± 0.1 °C are shown in Table 2, together with respective standard deviations. Despite the large distance between the $N^2$ nitrogen atom and R group, the R substituent seems to have a crucial role in p$K_{a1}$ values of sulfonamide derivatives. An enhanced effect in the chemical and biological properties that distinguish different sulfonamide-based drugs should be expected if -NH- substitution takes place due to its proximity to the leaving proton. Thus, the role of different R groups attached to the amide nitrogen atom should be equally inspected.

The p$K_1$ values associated with the amino group for the compounds studied were smaller than those generally observed with aniline derivatives in water[41,42] (*e.g.*, aniline in water has a p$K$ = 4.60). This increase in acidity can be attributed to an electron-withdrawing sulfone group in the para position. On the other hand, the p$K_{a2}$ values in line with the amide nitrogen atom of sulfonamide derivatives were smaller than the dissociation constant of sulfonilamide, p$K_a$ = 10.1.[43] This increase in acidity can be explained by a resonance and inductive effect on the dissociation constants because the structures of the sulfonamide

**Table 2.** p$K_a$ values of studied compounds in 15, 30, 40 and 50% v/v MeCN-water binary mixture by using LC methodology

| Compounds | Methods | MeCN-water binary mixtures | | | |
|---|---|---|---|---|---|
| | | 15% (v/v) | 30% (v/v) | 40% (v/v) | 50% (v/v) |
| Sulfadiazine | LC | 2.06 (0.28) | 2.03 (0.13) | 2.05 (0.18) | – |
| | | 6.86 (0.04) | 7.36 (0.01) | 7.86 (0.15) | 8.04 (0.02) |
| | LC-DAD | 2.16 (0.08) | 1.86 (0.05) | 2.03 (0.21) | 2.49 (0.04) |
| | | 6.65 (0.02) | 7.05 (0.05) | 7.15 (0.05) | 7.45 (0.09) |
| Sulfathiazole | LC | 2.18 (0.09) | 1.84 (0.08) | – | – |
| | | 7.38 (0.08) | 7.63 (0.01) | 7.84 (0.15) | 8.02 (0.02) |
| | LC-DAD | 2.28 (0.12) | 1.57 (0.11) | 2.18 (0.05) | 2.81 (0.04) |
| | | 7.31 (0.03) | 7.62 (0.06) | 7.78 (0.06) | 7.99 (0.12) |
| Sulfamerazine | LC | 2.16 (0.05) | 1.82 (0.25) | 2.03 (0.13) | – |
| | | 7.30 (0.06) | 7.80 (0.06) | 8.08 (0.01) | 8.18 (0.08) |
| | LC-DAD | 2.06 (0.10) | 1.80 (0.06) | 2.36 (0.07) | 2.84 (0.03) |
| | | 7.10 (0.01) | 7.48 (0.06) | 7.90 (0.05) | 8.24 (0.09) |
| Sulfamethazine | LC | 2.17 (0.09) | 2.05 (0.08) | 1.96 (0.16) | – |
| | | 7.47 (0.18) | 8.11 (0.04) | 8.27 (0.10) | 8.48 (0.05) |
| | LC-DAD | 2.06 (0.07) | 1.75 (0.08) | 2.26 (0.13) | 2.80 (0.03) |
| | | 7.36 (0.10) | 8.07 (0.10) | 8.32 (0.10) | 8.61 (0.09) |
| Sulfamonomethoxine | LC | 2.35 (0.55) | 2.07 (0.05) | – | – |
| | | 6.55 (0.09) | 6.91 (0.01) | 7.22 (0.16) | 7.42 (0.05) |
| | LC-DAD | 2.16 (0.25) | 1.94 (0.08) | 2.14 (0.06) | 2.89 (0.03) |
| | | 6.32 (0.04) | 6.42 (0.10) | 6.54 (0.06) | 6.87 (0.07) |
| Sulfadoxine | LC | 2.19 (0.23) | 2.06 (0.11) | – | – |
| | | 6.46 (0.02) | 6.94 (0.02) | 7.32 (0.09) | 7.61 (0.08) |
| | LC-DAD | 2.08 (0.23) | 2.06 (0.10) | 2.11 (0.05) | 2.79 (0.04) |
| | | 6.06 (0.02) | 6.54 (0.09) | 6.13 (0.14) | 6.32 (0.08) |
| Sulfamethaxazole | LC | – | 1.52 (0.15) | – | – |
| | | 6.32 (0.02) | 6.65 (0.03) | 6.91 (0.10) | 7.40 (0.04) |
| | LC-DAD | 1.98 (0.03) | 2.02 (0.08) | 2.41 (0.10) | 2.84 (0.03) |
| | | 5.95 (0.09) | 6.35 (0.06) | 6.65 (0.06) | 7.07 (0.04) |

derivatives are formed when a heterocyclic molecule is substituted for hydrogen of the sulfamine group. As it can be seen in Table 2, the p$K_{a1}$ values for some compounds in 40 and 50% v/v MeCN media could not be calculated from retention factors due to the insufficient retention times. However, the p$K_{a1}$ values of studied compounds could be calculated by LC-PAD methodology, because the absorbance spectra at the maximum of chromatographic peaks, obtained with a diode array detector provide adequate data and has been utilized for calculations.

It is known that one of the most important factor determining equilibrium constants is the reaction medium. The variation of the p$K_{a2}$ values of SAs *versus* the mole fraction of MeCN, $X_{MeCN}$, in the MeCN-water mixtures is presented in Figure 4. The equations between p$K_{a2}$ values and mole fraction of organic modifier are shown in Table 3. The different ways in which p$K$ values change might be explained by the fact that the dissociation process is ruled by electrostatic interactions as well as by specific solute-solvent interactions.

It has been found that in several water-organic binary solvent mixtures p$K_a$ values of a given substance show a linear relationship with the mole fraction of organic solvent.[37] This is indicated by the following expression

$$pK_{a,j} = j\Delta pK + pK_{a,w} \quad (5)$$

where p$K_{a,w}$ indicates the dissociation constant in water, j the mole fraction of organic solvent, $\Delta pK$ the slope of the linear relationship, and p$K_{a,j}$ the p$K_a$ at the corresponding composition. p$K$ values reported in the literature for water are shown in Table 4 for comparison together with the values obtained from equation 5. As it can be deduced from Table 4, the difference between the values determined in the present work and the values determined by others is minor and it could be expected from the difference in conditions and methods employed.

The p$K_a$ values of SAs obtained in MeCN-water binary mixtures increase with percentage of MeCN. These variations could be explained by the fact that there is

**Table 3.** The equations between p$K_{a2}$ values and mole fractions of organic modifier

| Compounds | Equation | Regression coefficient |
|---|---|---|
| Sulfadiazine | y = 6.183x* + 6.565 | R = 0.979 |
| Sulfathiazole | y = 3.268x + 7.207 | R = 0.997 |
| Sulfamerazine | y = 4.488x + 7.139 | R = 0.958 |
| Sulfamethazine | y = 4.931x + 7.313 | R = 0.953 |
| Sulfamonomethoxine | y = 4.478x + 6.326 | R = 0.992 |
| Sulfadoxine | y = 5.878x + 6.165 | R = 0.994 |
| Sulfamethaxazole | y = 5.381x + 5.980 | R = 0.993 |

* represent the mole fraction of organic modifier (MeCN).

preferential solvation in these media that is related to the structural features of these binary mixtures. Preferential solvation in MeCN-water mixtures produces lower $K_a$ values than those expected when the preferred solvent is water. The composition of the immediate surroundings of a solute may differ from the composition of the bulk mixture. Preferential solvation is attributable to an excess or deficiency of molecules of one of the solvents in these surroundings.[44] If the solute displays no preference for the solvent molecules, the solvent composition in the primary coordination shell, in the immediate neighborhood of the solute, is the same as that in the bulk. The deviation from the ideal dependence on the composition of the mixtures indicates that the solvent composition in the neighborhood of the solute may be different from that in the bulk.

As discussed above, the data shown in Table 2 clearly illustrate the important influence of the nature of the solvent on the dissociation reaction of studied compounds. It has been shown that the solvating ability and dielectric constant of the solvent play an important role in dissociation reactions.[45] Water is a solvent of high solvating ability (*i.e.* donor number DN = 33.0 and dielectric constant ε = 78) which can dissociate the acid and stabilize the produced anion and hydrogen ion. Thus, it is expected that addition of MeCN with lower donor number and dielectric constant (DN = 14.0, ε = 36.0) to water decreases the extent of interaction of the acid anion and the proton with solvent, and this decreases the acidity constant of the compound. It is interesting to note that there is actually a linear relationship between the p$K_{a2}$ values of the second dissociation step (that of the first step slightly changes, those of the second steps increase) and the mole fraction of MeCN ($X_{MeCN}$) in the binary mixtures that are shown in Figure 4.

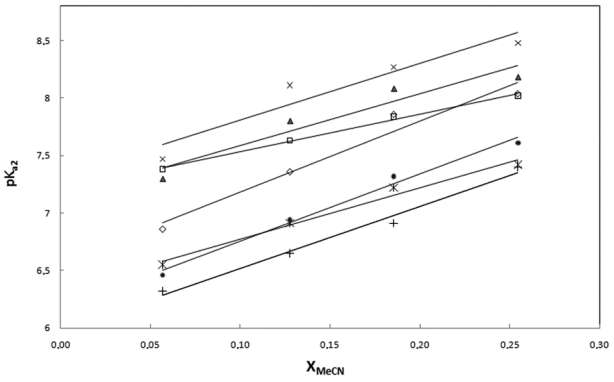



**Figure 4.** Plot of p$K_{a2}$ values against mole fraction of MeCN in the binary mixtures. (◊) sulfadiazine, (□) sulfathiazole, (▲) sulfamerazine, (×) sulfamethazine, (ж) sulfomonomethoxine, (●) sulfadoxine, (▶) sulfomethoxazole.

**Table 4.** p$K_a$ values reported of sulfonamides in water and calculated by equation 5

| Compounds | Method | p$K_{a2}$ | Background | p$K_{a2}$ (this work) | Ref. |
|---|---|---|---|---|---|
| sulfadiazine | CE | 6.28 | citrate buffer | 6.57 | 25 |
| | CE | 6.43 | buffer | | 27 |
| | LC | 6.6 | phosphate buffer | | 26 |
| sulfathiazole | Potentiometry | 7.11 ± 0.04 | 0.01-0.05 mol $L^{-1}$ $NaClO_4$ | 7.21 | 18 |
| | CE | 7.24 | buffer | | 27 |
| sulfamerazine | CE | 6.77 | citrate buffer | 7.14 | 25 |
| | Potentiometry | 6.90 ± 0.05 | 0.01-0.05 mol $L^{-1}$ $NaClO_4$ | | 18 |
| sulfamethazine | CE | 7.65 | buffer | 7.31 | 27 |
| sulfamono-methoxine | CE | 6.03 | buffer | 6.33 | 27 |
| | CE | 5.96 | citrate buffer | | 25 |
| sulfadoxine | LC | 6.1 | phosphate buffer | 6.17 | 26 |
| sulfamethoxazole | Potentiometry | 5.60 ± 0.04 | 0.01-0.05 mol $L^{-1}$ $NaClO_4$ | 5.98 | 18 |
| | CE | 5.65 | buffer | | 27 |
| | CE | 5.57 | citrate buffer | | 25 |
| | LC | 5.9 | phosphate buffer | | 26 |

It has been reasonably assumed that preferential solvation of the charged particles by water is mainly responsible for such a monotonic dependence of the acidity constants of SAs on the solvent composition. It is known that the dissociation of an uncharged acid in a solvent requires the separation of two ions of opposite charges. The work required to separate these charges is inversely proportional to the dielectric constant of the solvent. The energy required for dissociation is supplied by solvation of the ions, and also the proton transfer from acid to the solvent molecule supplies an additional energy. If the dielectric constant and the solvating ability of the solvent are decreased, more energy will be required to separate the anion and cation, and consequently the extent of dissociation of the acid will be lowered. Therefore, the chance in the p$K_a$ of first step and the increase in the second step are due to increasing the mole fraction of MeCN in the binary mixed solvent.

Furthermore, the first dissociation equilibrium (p$K_1$) regarding the $N^2$ nitrogen atom is the dissociation constant for equilibrium between the positively charged, unionized amino group and its electrically neutral conjugate base. Thus, when the amino group is ionized, there is no change in the number of charges involved in the process ($H_2B^+ \rightleftharpoons HB + H^+$). Therefore, a change in the polarity of the medium has a minor influence on the dissociation process, which depends only on the solvation of the different species by the solvents of the mixture, and the variation of the dissociation constant with the MeCN content is not linear. The changes in the p$K_{a1}$ values of sulfonamides in range of 40 and 50 % v/v of MeCN are greater. This is due to the fact that these dissociation constants are dependent on solute-solvent interaction effects, and these effects vary with the structural features of the mixtures. In these media, the solute interacts with solvent more strongly than with the other; the solute will be preferentially solvated by the former.[46]

## Conclusions

In this study, we distinguish the behavior of acidity constants of SAs in water-MeCN systems at 25 °C by LC and spectrophotometric data. The results obtained in this work indicate that the LC and LC-PDA methodology is a useful procedure in the determination of dissociation constants from chromatographic data: it allows two kinds of data sets (k/pH) and (A/pH) to be obtained, and these can be used for independent p$K_a$ determination. Therefore, a comparison can be made between these two procedures to explore and understand the obtained results; this is important because both data sets are obtained in the same experimental run.

Moreover, the results show that the p$K_a$ values of SAs are influenced by the percentages of organic solvent added to the solutions. The variation of p$K_a$ values with the mole fraction of MeCN is different for each substance although, in general, p$K_1$ values in line with the anilinium ion slightly changes up to 50 % (v/v) of MeCN, whereas p$K_2$ values in line with the sulfonamide increase. The different ways in which p$K$ values change might be explained by the fact that the dissociation process is ruled by electrostatic interactions as well as by specific solute-solvent interactions.

## Acknowledgments

Financial support of this project by SDU-BAP (project No. 962-D-04) is gratefully acknowledge. Also, the authors greatly acknowledge Dr. Jose L. Beltran from Universitat de Barcelona for kindly providing the spectral data processing software, STAR. Authors are thankful to Anadolu University BIBAM and Dr. Zerrin SALTAN for their great assistance during the determination process.

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*Submitted: December 3, 2009*
*Published online: July 8, 2010*