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Evaluation of the effect of organic modifier on p*K* values of diuretics in mobile phases used in LC

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Abstract

The dissociation p*K* values of a series of diuretics in 10, 30, 40, 50 and 70% (w/w) acetonitrile–water mixed solvents at 25°C were determined according to the criteria endorsed by IUPAC. The series of diuretics chosen includes compounds with differences in molecular structures and physico-chemical properties. Acidic compounds (loop diuretics, such as furosemide, bumetanide and ethacrynic acid), weakly acidic (thiazides, such as chlorthiazide and trichlormethiazide), neutral (aldosterone antagonists, such as canrenone) and basic compounds (potassium-sparing diuretics, such as amiloride and triamterene) were all considered. The variation of the p*K* values obtained over the whole composition range studied can be explained by taking into account the preferential solvation of ionizable compounds in acetonitrile–water mixtures. Moreover, in order to obtain p*K* values in any of the unlimited number of possible binary solvent acetonitrile–water mixtures, relationships between p*K* values and different bulk properties were examined, and the linear solvation energy relationships method (LSER) was applied to study the correlations of p*K* values with the solvatochromic parameters π^*, α and β of acetonitrile–water mixtures. The equations obtained allowed calculation of the p*K* values of diuretics in any acetonitrile–water mixtures up to 70% (w/w) and thus permitted the acid–base behaviour of these important substances in the widely used acetonitrile–water media to be known. © 1998 Elsevier Science B.V.

Keywords: Acetonitrile–water mixtures; Diuretics; Linear solvation energy relationships; p*K* values; Preferential solvation

1. Introduction

Diuretics are widely used therapeutically for hypertension and edema resulting from cardiac or

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renal failure. These agents typically increase the urine flow and the net renal excretion of both sodium and water (Bridges and Chasseaud, 1983).

The determination of diuretics in biological fluids is interesting due to the potential role of therapeutic drug monitoring in optimizing patient care and also because in recent years diuretics have been misused and abused in sports that involve weight categories, either to reduce weight prior to competition or to dilute the urine specimen by producing larger volumes of urine in an attempt to evade drug detection. Diuretics have also been used in sport to control water retention, which is one of the most frequent adverse effects of anabolic steroids.

Diuretics are generally excreted in unchanged form to a high degree (Ventura et al., 1993). Therefore, procedures to screen for diuretics in biological fluids can be designed to detect the suspected parent compounds.

Most of the procedures described in the literature are high-performance liquid chromatographic-based methods using acetonitrile–water mixtures as mobile phase (Cooper et al., 1989; Park et al., 1990b; Herráez-Hernández et al., 1992; Ventura et al., 1993; Campins-Falcó et al., 1994).

However, the group of diuretics includes compounds with wide differences in physico-chemical properties. The heterogeneity of diuretics, their varied chemical structures, functional groups and pK values, causes problems in developing a common screening procedure.

In previous works, the use of solvatochromic parameter values of aqueous–organic mixtures used in liquid chromatography (LC) was studied in order to predict the retention of a series of quinolones (Barbosa et al., 1996), peptides (Barbosa et al., 1995b) and anabolic steroids (Barrón et al., 1995, 1996) in LC. However, in order to predict the influence of different mobile phase compositions on the selectivity of ionizable compounds, one should also determine how the concentration of the organic modifier influences the ionization equilibria. Since the pK values of diuretics are different, adjustment of pH has a great effect on separation selectivity.

In the specific case of acetonitrile–water mixtures, which are the most widely used mobile phases in LC, addition of acetonitrile, a slightly acidic and basic solvent ($pK_{ap} = 33.6$) (Barbosa and Sanz-Nebot, 1989) increases the magnitude and range of ionization constants (Barbosa and Sanz-Nebot, 1991; Barbosa et al., 1994) and not in a simple manner (Barbosa et al., 1997a). Consequently, the pH required to maximize the retention of solutes by ion suppression could be different from expected, taking into account the pK values obtained in aqueous media. Unfortunately, the pK-value data in acetonitrile–water mixtures are scarce (Barbosa et al., 1994, 1997b,c). Thus, the study of the acid–base behaviour of analytes in acetonitrile–water mixtures could be very important in predicting the influence of pH on retention and selectivity in LC.

In a previous work (Barbosa and Sanz-Nebot, 1995), the assignment of reference pH-values to primary standard buffer solutions for the standardization of potentiometric sensors in acetonitrile–water mixtures was established. Thus, pH measurements in these media can be performed in a manner similar to those in water.

The present study concerns the determination of the pK values of a series of diuretics in 10, 30, 40, 50 and 70% (w/w) acetonitrile–water mixtures according to the rules and procedures endorsed by IUPAC (Rondinini et al., 1987). The series of diuretics chosen includes compounds with differences in molecular structures and physico-chemical properties. Acidic compounds (loop diuretics, such as furosemide, bumetanide and ethacrynic acid), weakly acidic (thiazides, such as chlorthiazide and trichlormethiazide), neutral (aldosterone antagonists, such as canrenone) and basic compounds (potassium-sparing diuretics, such as amiloride and triamterene) were all considered. The variation of the pK values obtained over the whole composition range studied can be explained by taking into account the preferential solvation of ionizable compounds in acetonitrile–water mixtures. Preferential solvation produces lower pK values than expected if the 'preferred' solvent has the highest relative permittivity (Barbosa et al., 1997a). Moreover, in order to obtain pK values in any of the unlimited number of possible

binary solvent acetonitrile–water mixtures, relationships between pK values and different bulk properties (such as dielectric constant) were examined, and the linear solvation energy relationships method (LSER) (Kamlet et al., 1983) was applied to study the correlations of pK values with the solvatochromic parameters of acetonitrile–water mixtures. The equations obtained allowed calculation of the pK values of diuretics in any acetonitrile–water mixtures up to 70% (w/w), and thus permitted the acid–base behaviour of these important substances in the widely used acetonitrile–water media to be known.

2. Experimental

2.1. Apparatus

The electromotive force (emf) values of the potentiometric cell were measured with a CRISON micropH 2002 equipped with a CRISON-INGOLD 102623015 glass electrode and an Ag/AgCl reference electrode prepared according to the electrolytic method (Barbosa and Sanz-Nebot, 1991) and directly immersed in the solution to avoid residual liquid junction potential. The glass electrode was stored in water when not in use and soaked for 15–20 min in acetonitrile–water before potentiometric measurement.

The reference electrode was stable for 3 months of continuous work and during the whole time the standard potential in every solvent, E^0, remained essentially constant (standard deviation, $s < 0.6$ mV). The standardization of the electrode system was carried out each time the solvent medium was changed, and the constancy of E^0 values was ensured by continual surveillance by means of periodic calibrations. The stabilization criterion for emf readings was 0.2 mV within 2 min; if the stabilization was not achieved after 10 min, more titrant was added. The system gave stable, reproducible potentials within 4 min. The cell was thermostatted externally at 25 ± 0.1°C. The titrant was added from a METROHM Dosimat 665 autoburette. The potentiometric system was automatically controlled with a PC microcomputer.

3. Reagents

Analytical reagent grade chemicals were used unless indicated otherwise.

All solutions were prepared by mixing double-distilled freshly boiled water whose conductivity did not exceed 0.05 μS cm^{-1} and acetonitrile (Merck, chromatography grade).

Potassium hydroxide (0.1 M) working solutions were obtained by diluting a concentrated solution 1 M (Merck), prepared with an ion-exchange resin to avoid carbonation, and were standardized volumetrically against potassium hydrogen phthalate. Because of the low solubility of potassium hydroxide when using 70% (w/w) acetonitrile–water, the concentration of KOH solution in this medium was 0.02 M. HCl 0.05 M solutions were prepared by diluting the commercial reagent (Merck, 25%).

Ethacrynic acid, furosemide, bumetanide, canrenoic acid (potassium salt), chlorothiazide, trichlormethiazide, triamterene and amiloride (hydrochloride) were supplied by Sigma. Canrenoic acid solutions were obtained from their potassium salt by adding the stoichiometric volume of HCl. Trivial names and molecular structures are shown in Fig. 1.

3.1. Procedures

The pK values of the diuretics were determined from titration of appropriate solutions of acid species in 10, 30, 40, 50 and 70% (w/w) acetonitrile–water mixtures using potassium hydroxide solutions in the same mixture as titrant and approximately 7×10^{-3} M KCl solution for the correct response of the electrode system. For basic diuretics, such as amiloride and triamterene, an excess of HCl solution was added so that these diuretics were fully protonated at the beginning of the titration.

The pK values were obtained from systematic measurements of the emf of the cell:

$$\mathrm{Pt|Ag|AgCl|HA + A + KCl\ in\ acetonitrile\text{–}water|}GE$$

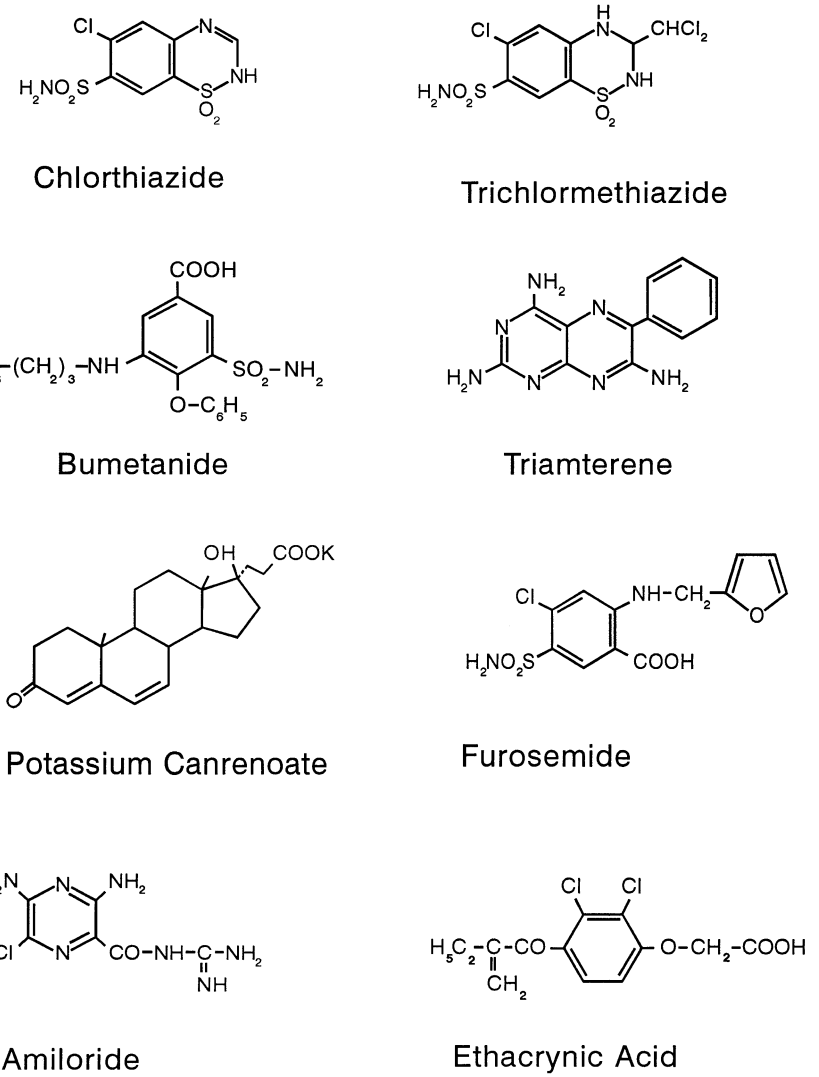


Fig. 1. Molecular structures and trivial names for diuretics.

where HA and A are the acidic and basic species involved in the dissociation equilibrium studied. The emf, E, of this cell is directly related to the activities of the hydrogen and chloride ions in solution:

$$E = E^0 + g \log(a_{H+} \cdot a_{Cl-})$$

where g is the Nernst coefficient (RT/F) and E^0, the standard emf of the cell, was determined as in a previous study (Barbosa and Sanz-Nebot, 1991).

The dissociation constant for these species could be expressed as:

$$K = \frac{c_A \gamma_A c_{H+} \gamma_{H+}}{c_{HA} \gamma_{HA}}$$

Then the equation which allows pK calculation is:

$$pK = \frac{E^0 - E}{g} + \log \frac{c_{HA} \gamma_{HA} c_{Cl-} \gamma_{Cl-}}{c_A \gamma_A}$$

where c_{HA} and c_A are the molar concentrations of acidic and basic species, c_{Cl-} is the molar concen-

tration of the mixed electrolyte KCl and γ_x the molar activity coefficient of species x.

In cases of $pK < 5$, computation of c_{HA} and c_A values required knowledge of c_{H+}, which is a function of the molar activity coefficient γ, which could be calculated using the Debye–Hückel equation:

$$p\gamma_{Cl-} = \frac{AI^{1/2}}{(1 + a_0 BI^{1/2})}$$

where A and B are the Debye–Hückel constants, a_0 is the ion size parameter in the solvent mixture and I is the ionic strength. Values of A and a_0B at 25°C at different percentages of acetonitrile in all mixtures with water were reported in previous works (Barbosa and Sanz-Nebot, 1991, 1994).

Calculation of $p\gamma_{Cl-}$ requires a knowledge of the ionic strength I of the HA + A + KCl mixed electrolyte solution. I is a function of the c_{H+} which is expressed by:

$$pc_{H+} = \frac{E^0 - E}{g} - pc_{Cl-} - p(\gamma_{H+}\gamma_{Cl-})$$

Thus, determination of pK values requires an iterative cycle for each point of the potentiometric titration at which E is measured. The calculation begins with $I = c_A + c_{Cl-}$ and γ_{Cl-} values are obtained using the Debye–Hückel equation for their subsequent use to obtain pc_{H+} values and better value of I and so on, until constancy of I values is obtained.

The difference between the two ionization constants of amiloride is not very high and the second ionization equilibrium of the diuretics took place in very alkaline conditions. Thus, the calculations were carried out making use of the program written in PASCAL, PKPOT (Barbosa et al., 1995a). The least-squares PKPOT program allows the determination of thermodynamic acid–base constants, in aqueous and non-aqueous media, taking into account the activity coefficients of the species. These mathematical procedures also permit the determination of pK values in overlapping ranges ($pK_i - pK_j < 2$) and dissociation constants in very alkaline conditions. The procedures are based on the postulation of a chemical model, i.e. of an initial set of species defined by their stoichiometric coefficients and formation constants, which are then refined in the least-squares minimization.

4. Results and discussion

Emf measurements for the cell were made at different concentrations of acidic, HA, and basic, A, species of diuretics in 10, 30, 40, 50 and 70% (w/w) acetonitrile–water solvent mixtures. For each diuretic in each solvent mixture studied, from four to six series of measurements were performed, for a total of 3450 independent measurements over the solvent interval explored. To simplify the tabulation and as an example, one series of measurements for one titration of chlorthiazide in 70% (w/w) acetonitrile–water using KOH solution in the same solvent as titrant is given in Table 1. The calculations were made using the program written in PASCAL, the PKPOT previously reported (Barbosa et al., 1995a).

Table 2 shows the ionization constants values determined for the series of eight diuretics studied in 10, 30, 40, 50 and 70% (w/w) acetonitrile–water mixtures and the respective standard deviation, s, together with the previously mentioned pK values in water (Moffat et al., 1986). Values of bumetanide in 10% (w/w) and canrenoic acid in 10 and 30% (w/w) mixtures were not determined because of the non-solubility of these substances in these media.

As is shown in Table 2, ethacrynic acid has only one pK value corresponding to the dissociation of the carboxylic acid, whereas furosemide, bumetanide, chlorthiazide and trichlormethiazide have two acid–base functional groups. Values obtained for loop diuretics such as furosemide and bumetanide agree well with the protolytic equilibrium of carboxylic acid which takes place in acid media and the sulphonamide group's dissociation under alkaline conditions (Sistovaris et al., 1991) (Fig. 2). The pK_2 values of chlorthiazide and trichlormethiazide are similar to pK_2 values of loop diuretics and can be assigned to the dissociation of the sulphonamide group. Thus, pK_1 of these diuretics could be assigned to the other ionizable hydrogen, as shown in Fig. 2.

Table 1
The pK values of chlorthiazide in 70% (w/w) acetonitrile–water

V_0 (ml)	V_e (ml)		[KCl] (M)	[KOH] (M)	E^0 (mV)		
20	1.43	2.86	6.14×10^{-3}	0.0196	510.95		
V (ml)	E (mV)	$[K^+]$	$[Cl^-]$	$[H^+]$	$[H_2A]$	$[HA^-]$	$[A^{2-}]$
0.10	−51.10	6.21×10^{-3}	6.11×10^{-3}	7.27×10^{-8}	1.28×10^{-3}	1.44×10^{-4}	6.74×10^{-9}
0.25	−71.80	6.31×10^{-3}	6.06×10^{-3}	3.20×10^{-8}	1.13×10^{-3}	2.88×10^{-4}	3.08×10^{-8}
0.30	−76.80	6.34×10^{-3}	6.05×10^{-3}	2.63×10^{-8}	1.08×10^{-3}	3.35×10^{-4}	4.38×10^{-8}
0.40	−85.50	6.40×10^{-3}	6.02×10^{-3}	1.86×10^{-8}	9.76×10^{-4}	4.30×10^{-4}	7.96×10^{-8}
0.50	−93.26	6.47×10^{-3}	5.99×10^{-3}	1.37×10^{-8}	8.76×10^{-4}	5.23×10^{-4}	1.32×10^{-7}
0.60	−100.50	6.53×10^{-3}	5.96×10^{-3}	1.04×10^{-8}	7.77×10^{-4}	6.15×10^{-4}	2.06×10^{-7}
0.70	−107.70	6.60×10^{-3}	5.93×10^{-3}	7.88×10^{-9}	6.78×10^{-4}	7.07×10^{-4}	3.12×10^{-7}
0.75	−111.30	6.63×10^{-3}	5.92×10^{-3}	6.88×10^{-9}	6.30×10^{-4}	7.52×10^{-4}	3.80×10^{-7}
0.80	−114.40	6.66×10^{-3}	5.90×10^{-3}	5.99×10^{-9}	5.81×10^{-4}	7.97×10^{-4}	4.64×10^{-7}
0.91	−123.50	6.73×10^{-3}	5.87×10^{-3}	4.37×10^{-9}	4.75×10^{-4}	8.96×10^{-4}	7.16×10^{-7}
1.00	−131.20	6.78×10^{-3}	5.85×10^{-3}	3.29×10^{-9}	3.89×10^{-4}	9.75×10^{-4}	1.04×10^{-6}
1.06	−137.00	6.82×10^{-3}	5.83×10^{-3}	2.67×10^{-9}	3.33×10^{-4}	1.03×10^{-3}	1.35×10^{-6}
1.12	−143.50	6.85×10^{-3}	5.81×10^{-3}	2.12×10^{-9}	2.77×10^{-4}	1.08×10^{-3}	1.79×10^{-6}
1.21	−155.70	6.91×10^{-3}	5.79×10^{-3}	1.38×10^{-9}	1.93×10^{-4}	1.16×10^{-3}	2.94×10^{-6}
1.30	−172.30	6.96×10^{-3}	5.77×10^{-3}	7.59×10^{-10}	1.13×10^{-4}	1.23×10^{-3}	5.71×10^{-6}
1.36	−187.80	7.00×10^{-3}	5.75×10^{-3}	4.09×10^{-10}	6.26×10^{-5}	1.27×10^{-3}	1.10×10^{-5}
1.42	−208.40	7.03×10^{-3}	5.73×10^{-3}	1.61×10^{-10}	2.50×10^{-5}	1.29×10^{-3}	2.82×10^{-5}
1.45	−219.70	7.05×10^{-3}	5.73×10^{-3}	9.93×10^{-11}	1.53×10^{-5}	1.28×10^{-3}	4.56×10^{-5}
1.51	−239.10	7.09×10^{-3}	5.71×10^{-3}	4.81×10^{-11}	7.15×10^{-6}	1.24×10^{-3}	9.13×10^{-5}
1.54	−246.50	7.10×10^{-3}	5.70×10^{-3}	3.71×10^{-11}	5.39×10^{-6}	1.21×10^{-3}	1.16×10^{-4}
1.60	−257.30	7.14×10^{-3}	5.69×10^{-3}	2.47×10^{-11}	3.43×10^{-6}	1.16×10^{-3}	1.68×10^{-4}
1.63	−262.40	7.15×10^{-3}	5.68×10^{-3}	2.09×10^{-11}	2.84×10^{-6}	1.13×10^{-3}	1.93×10^{-4}
1.66	−267.00	7.17×10^{-3}	5.67×10^{-3}	1.81×10^{-11}	2.38×10^{-6}	1.10×10^{-3}	2.19×10^{-4}
1.72	−274.00	7.21×10^{-3}	5.65×10^{-3}	1.39×10^{-11}	1.75×10^{-6}	1.05×10^{-3}	2.71×10^{-4}
1.75	−277.10	7.22×10^{-3}	5.65×10^{-3}	1.24×10^{-11}	1.51×10^{-6}	1.02×10^{-3}	2.97×10^{-4}
1.78	−280.20	7.24×10^{-3}	5.64×10^{-3}	1.11×10^{-11}	1.32×10^{-6}	9.93×10^{-4}	3.23×10^{-4}
1.84	−285.80	7.27×10^{-3}	5.62×10^{-3}	9.11×10^{-12}	1.02×10^{-6}	9.38×10^{-4}	3.74×10^{-4}
1.87	−288.40	7.29×10^{-3}	5.62×10^{-3}	8.30×10^{-12}	9.00×10^{-7}	9.11×10^{-4}	3.99×10^{-4}
1.90	−290.50	7.31×10^{-3}	5.61×10^{-3}	7.58×10^{-12}	7.97×10^{-7}	8.84×10^{-4}	4.25×10^{-4}
1.93	−292.40	7.33×10^{-3}	5.60×10^{-3}	6.95×10^{-12}	7.07×10^{-7}	8.57×10^{-4}	4.50×10^{-4}
1.96	−294.00	7.34×10^{-3}	5.59×10^{-3}	6.38×10^{-12}	6.29×10^{-7}	8.30×10^{-4}	4.75×10^{-4}
1.99	−295.60	7.36×10^{-3}	5.58×10^{-3}	5.88×10^{-12}	5.60×10^{-7}	8.03×10^{-4}	5.00×10^{-4}
2.02	−297.10	7.38×10^{-3}	5.58×10^{-3}	5.42×10^{-12}	4.99×10^{-7}	7.77×10^{-4}	5.25×10^{-4}

$pK_1 = 8.23$; $pK_2 = 11.75$

V_0 is the initial volume of solution (ml), V_e the equivalence volumes, [KOH] the titrant concentration, [KCl] the initial KCl concentration, and E_0 the standard emf of the cell, previously standardized. For each volume of titrant added, V, the emf value, E, is measured, $[H_2A]$ is the concentration of acidic species, $[HA^-]$ the concentration of intermediate species, $[A^{2-}]$ the concentration of basic species, and $[K^+]$, $[Cl^-]$ and $[H^+]$ the concentrations of potassium, chloride and hydrogen ions, respectively, at each point of the titration.

The reaction of amiloride with hydrochloric acid is equimolar: from potentiometric titrations of amiloride hydrochloride solutions or amiloride with excess of HCl solutions with KOH, the same results are obtained. Thus, pK_1 corresponds to the deprotonation of the aliphatic amino group and pK_2 to the ionization of the amide functional group. Triamterene shows one dissociation equilibrium corresponding to the deprotonation of one amine functional group. The pK values of

Table 2
pK Values of diuretics in acetonitrile–water mixtures up to 70% (w/w) at 25°C

Diuretic		% (w/w) Acetonitrile					
		0	10	30	40	50	70
Ethacrynic acid	pK_1	3.5	3.75 (0.02)	4.10 (0.01)	4.45 (0.01)	4.83 (0.02)	5.79 (0.02)
Furosemide	pK_1	3.9	4.52 (0.04)	4.87 (0.02)	5.25 (0.03)	5.58 (0.01)	6.41 (0.02)
	pK_2	—	10.1 (0.09)	10.29 (0.1)	11.01 (0.07)	11.27 (0.1)	12.29 (0.05)
Bumetanide	pK_1	—	—	5.12 (0.01)	5.50 (0.01)	5.86 (0.03)	6.83 (0.01)
	pK_2	—	—	10.75 (0.1)	10.91 (0.2)	11.11 (0.2)	11.75 (0.3)
Canrenoic acid	pK_1	—	—	—	6.43 (0.01)	6.51 (0.02)	7.72 (0.01)
Chlorthiazide	pK_1	6.7	6.95 (0.01)	7.14 (0.03)	7.35 (0.01)	7.62 (0.01)	8.25 (0.04)
	pK_2	9.5	9.72 (0.04)	9.82 (0.2)	10.27 (0.5)	10.95 (0.2)	11.27 (0.6)
Trichlormethiazide	pK_1	—	7.30 (0.02)	7.93 (0.01)	8.30 (0.02)	8.83 (0.04)	9.46 (0.05)
	pK_2	—	10.0 (0.1)	10.62 (0.5)	11.35 (0.1)	11.61 (0.2)	12.44 (0.03)
Triamterene	pK_1	6.2	6.12 (0.03)	6.25 (0.03)	6.28 (0.04)	6.55 (0.01)	6.79 (0.04)
Amiloride	pK_1	—	8.37 (0.01)	8.90 (0.05)	9.04 (0.03)	9.28 (0.03)	9.67 (0.05)
	pK_2	—	10.57 (0.3)	10.93 (0.3)	11.56 (0.1)	12.34 (0.2)	12.76 (0.3)

Values in parentheses are standard deviation, $30 < n < 60$). Values of dissociation constants of diuretics in water are obtained from the literature (Moffat et al., 1986).

triamterene were obtained from titrations with KOH of triamterene and HCl solutions. The carboxylic functional group of canrenoic acid is responsible for its pK value.

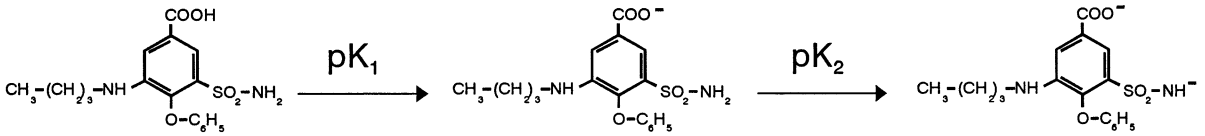
The few pK values of diuretics in the literature correspond to their values in water and are shown in Table 2 (Moffat et al., 1986). However, some other data in the literature, such as $pK_1 = 3.6$ and $pK_1 = 5.2$ (Moffat et al., 1986) for bumetanide and $pK_1 = 8.6$ for trichlormethiazide, differ considerably, and the methods used for their determination are not adequately described (temperature, solvent media, ionic strength, etc.) and are omitted from Table 2.

Consideration of the data in Table 2 shows that the extrapolation of pK values in water to acetonitrile–water mixtures is not linear. This table shows that it is difficult to interpret the variations of pK_1 and pK_2 of diuretics according to the percentage of acetonitrile in the mixtures.

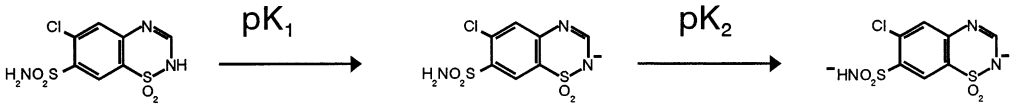
However, pK values corresponding to the dissociation of an acid functional group such as carboxylic, sulphonamide and amide vary in line with the percentage of acetonitrile, differently from the protonation behaviour of amine nitrogens of amiloride and trianterene. For dissociation of acid functional groups, differences as large as 2 units in pK values are obtained by changing the percentage of acetonitrile from 10% (w/w) to 70% (w/w), while pK values corresponding to the deprotonation of amine nitrogens show minor changes in the same range of solvents.

The variation of the pK_1 values of diuretics with dielectric constant is presented in Table 3. Canrenoic acid is omitted because only three values can be determined. The variation is different for each substance although, in general, the pK values increase when the acetonitrile content increases. However, the correlation coefficients of amiloride and trianterene are low compared with the good correlation coefficients obtained for the other diuretics.

Dissociation of a compound in a solvent S is governed by electrostatic interaction, as well as by specific solute–solvent interactions (solvation effects). In the dissociation of neutral or anions acids, charges are created ($HA \leftrightarrow H^+ + A^-$ or $HA^- \leftrightarrow H^+ + A^{2-}$) and the dissociation process is disturbed when the dielectric constant of the medium changes with the changes in acetonitrile content. In these cases the electrostatic interaction overwhelms the specific solvation: and for a series of solvents with similar acidity, the change in the dissociation constant can mainly be attributed to



Bumetanide



Chlorthiazide

Fig. 2. Protolytic equilibria of acidic diuretics.

the change in dielectric constant. Thus, the following expression can be written (Barbosa et al., 1994, 1997b): $pK = A + B/\epsilon$. Hence, the correlations between pK values, corresponding to the dissociation of carboxylic and sulphonamide groups, with the reciprocal of the dielectric constant, $1/\epsilon$, of the acetonitrile–water mixtures, are linear with correlation coefficients greater than 0.99 (Table 3). However, in dissociation of a monocharged cation acid (such as the amine ions of amiloride and trianterene), there is no change in the number of charges ($HA^+ \leftrightarrow H^+ + A$) and the change on the dielectric constant of the medium does not affect the dissociation process. In this instance, the dissociation depends only on the solvation of the different species by the solvents of the mixture. Hence, the correlation of pK_1 of amiloride and trianterene with $1/\epsilon$ is poor (Table 3).

In acetonitrile–water mixtures up to 70% (w/w), ϵ^{-1} versus x (the molar fraction of acetonitrile) are related by means of $\epsilon^{-1} = 1.26 \times 10^{-2} + 1.73 \times 10^{-2}x$, 0.9999 being the standard deviation of this equation. Thus, equation $pK = A + B/\epsilon$ becomes $pK = A' + B'x$.

The variation of pK_1 values of diuretics versus the mole fraction of acetonitrile, x, is given in Table 3. As expected, the pK_1 of ethacrynic acid, furosemide, bumetanide, chlorthiazide and trichlormethiazide are linearly correlated with x, with the correlation coefficient greater than 0.99; while the correlation coefficient of pK_1 of amiloride and trianterene with x is poor. In Table 3, the obtained pK_2 values of diuretics are not considered because the corresponding equilibria take place under very alkaline conditions and the errors are greater than at other pH values (Table 2).

Although pK_1 and pK_2 values of diuretics obtained in acetonitrile–water mixtures increase with the percentage of acetonitrile, these pK values are lower than the expected ones considering the high pK values expected in the neat solvent acetonitrile (Kolthoff, 1974). The change of pK values of diuretics in acetonitrile–water mixtures could be explained by the fact that preferential solvation by water exists in acetonitrile–water mixtures (Barbosa et al., 1997a) and is related to structural features of these mixtures (Easteal and Woolf, 1988). Preferential solvation in acetonitrile–water mixtures produces lower pK values than expected if the preferred solvent is water. The composition of the immediate surroundings of a solute may be different from the composition of the bulk mixture. Preferential solvation is attributable to an excess or a deficiency of

Table 3
pK Values vs. reciprocal of the dielectric constant, $1/\epsilon$, and vs. mole fraction, x, of acetonitrile–water mixtures

Diuretic	pK Against $1/\epsilon$	r	pK Against x	r
Ethacrynic acid	$pK_1 = 0.22 + 258/\epsilon$	0.996	$pK_1 = 3.46 + 4.54x$	0.997
Furosemide	$pK_1 = 1.28 + 239/\epsilon$	0.998	$pK_1 = 4.28 + 4.20x$	0.998
Bumetanide	$pK_1 = 0.86 + 279/\epsilon$	0.999 (4)	$pK_1 = 4.37 + 4.87x$	0.999 (4)
Chlorthiazide	$pK_1 = 4.66 + 166/\epsilon$	0.992	$pK_1 = 6.74 + 2.92x$	0.994
Trichlorthiazide	$pK_1 = 3.78 + 271/\epsilon$	0.991	$pK_1 = 7.19 + 4.75x$	0.990
Triamterene	$pK_1 = 4.92 + 87/\epsilon$	0.97	$pK_1 = 6.02 + 1.53x$	0.98
Amiloride	$pK_1 = 6.42 + 155/\epsilon$	0.98	$pK_1 = 8.38 + 2.72x$	0.98

Correlation coefficient r refers to five data points unless indicated otherwise.

molecules of one of the solvents in these surroundings (Marcus, 1989). If the solute has no preference between the solvent molecules, the solvent composition in the cybotactic zone, in the immediate neighbourhood of the solute, is the same as in the bulk. For such cases:

$$pK_S = x_1 pK_{S_1} + x_2 pK_{S_2}$$

where pK_S is the pK value in the mixture and pK_{S_1} and pK_{S_2} represent the pK values in acetonitrile and water, respectively. The deviation from the ideal dependence on the composition of the mixture indicates that the solvent composition in the neighbourhood of the solute may be different from that in the bulk. In acetonitrile–water mixtures there are three regions (Kovacs and Laarksonen, 1991; Marcus and Migron, 1991). On the water-rich side, there is a region in which the water structure remains more or less intact (Easteal and Woolf, 1988) and the acetonitrile molecules gradually occupy the cavities between water molecules. In the range $0.15 \leq x_{AN} \leq 0.75$, there are cluster of molecules of the same kind surrounded by regions where molecules of both kinds are near each other. In these regions, preferential solvation by water exists, which could explain the low increase of pK_1 and pK_2 values of diuretics when the percentage of acetonitrile increases. This is in accordance with the previously obtained values of preferential solvation, δ_W, of hydrogen ions by water in acetonitrile–water mixtures (Barbosa et al., 1997a). In these regions ($x_{AN} < 0.75$), the solutes are preferentially solvated by water and the variations of pK values are not great.

At $x_{AN} \geq 0.75$ the number of water clusters is low, and water–acetonitrile interactions that could be discounted in the middle range now become important. This may be considered as a region in which preferential solvation by water decreases (Barbosa et al., 1997a).

The pK values of diuretics in neat acetonitrile are not known, but pK values of citric acid were determined in previous studies (Barbosa et al., 1994, 1997b) over the whole composition range of acetonitrile–water mixtures. Figs. 3 and 4 show these pK values as a function of x_W, the bulk mole

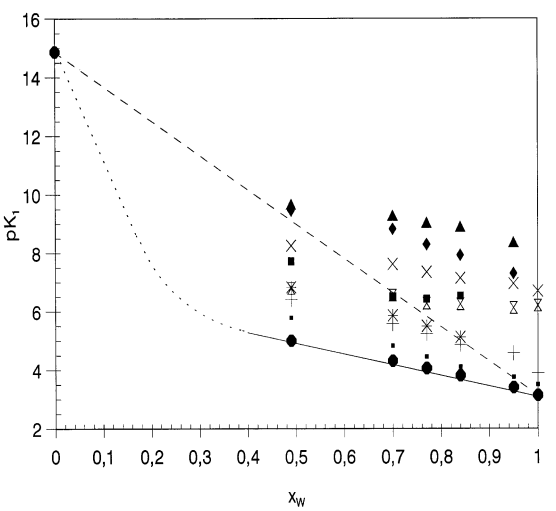


Fig. 3. The pK_1 vs. mole fraction of water, x_W, in acetonitrile–water mixtures. (■) Ethacrynic acid; (+) furosemide; (*) bumetanide; (■) canrenoic acid; (x) chlorthiazide; (◆) trichlormethiazide; (▲) amiloride; (X) triamterene; (●) citric acid. The dashed straight lines correspond to the ideal variation of the pK_1 values for the citric acid.

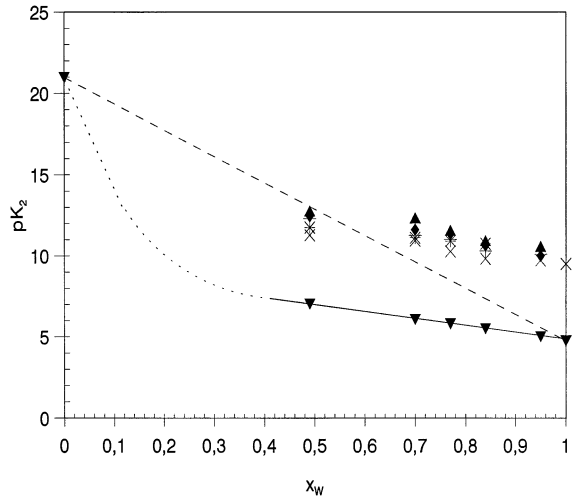


Fig. 4. pK_2 vs. mole fraction of water, x_W, in acetonitrile–water mixtures. (+) Furosemide; (*) bumetanide; (x) chlorthiazide; (◆) trichlormethiazide; (▲) amiloride; (▼) citric acid. The dashed straight lines correspond to the ideal variation of the pK_2 values for the citric acid.

fraction of water, where the dotted line represents the expected variation of the pK values between $x_{AN} \cong 0.5$ and pure acetonitrile solvent. Fig. 3 also shows the obtained pK_1 values of diuretics; and Fig. 4, pK_2 values. The slope of pK_1 values of acid diuretics versus x_W plot is greater than the pK_1 values of basic diuretics versus x_W plot in water-rich areas because of the influence of ϵ. The obtained pK_1 and pK_2 values change but are lower than the theoretical ones because of the preferential solvation by water; a concave variation of pK versus x_W may be expected with an inflection point at $x_W = 0.25$, where preferential solvation by water is maximal (Barbosa et al., 1997a).

On the other hand, it is not clear that solvatochromic parameters would be valid to act as stand-ins for generalized solutes in binary solvent mixtures with regard to properties they are supposed to measure. Preferential solvation in such mixtures may interfere more seriously with the ability of indicators to act as stand-ins for generalized solutes than in the case of single solvents. Progress has been made (Marcus and Migron, 1991; Migron and Marcus, 1991) and although this problem has not been solved unequivocally, these investigations provide significant evidence that the solvatochromic parameters seem to have general validity. It is therefore of interest to examine the linear solvation energy relationships (LSER) which explain any solute property varying with solvent composition as a linear combination of the microscopic parameters of solvent responsible. The Kamlet–Taft (Kamlet et al., 1983) expression states:

$$XYZ - (XYZ)_0 + a\alpha + b\beta + s\pi^*$$

where α, β and π^* are the microscopic parameters previously described, XYZ is the solute property, $(XYZ)_0$ the value of this property for the same solute in a hypothetical solvent for which $\alpha = \beta = \pi^* = 0$ and a, b and s are the susceptibilities of the solute property studied to changes in α, β and π^*, respectively. This equation can include additional terms or some of its terms can become equal to zero, depending on the property of the solute to be described (Kamlet and Taft, 1985). Values of the Kamlet–Taft solvatochromic parameters π^* (Cheong and Carr, 1988; Marcus and Migron, 1991), α (Park et al., 1990a; Marcus and Migron, 1991) and β (Marcus and Migron, 1991; Krygowski et al., 1985) for acetonitrile–water mixtures over the entire range of composition are known (Barbosa et al., 1997a).

Several attempts were made to find the best form of Kamlet–Taft equation to describe the variation of pK_1 and pK_2 values of diuretics in acetonitrile–water mixtures. Multiple regression analysis was applied to our pK data. All possible combinations of solvatochromic parameters, including Dimroth and Reichardt normalized parameter E_T^N, were checked. The best fit was obtained when the three solvatochromic parameters α, β and π^* were used, providing the general equations in Table 4. The high coefficient in the β terms compared with the α and π^* terms confirmed the main dependence of the pK_1 and pK_2 values of diuretics on the hydrogen bond accepting basicity of the solvent for the whole range of composition studied, up to 70% (w/w) of acetonitrile, in acetonitrile–water mixtures. Also, the s coefficients are negative for pK_1 and pK_2 values (Table 4), which means that an increase in the polarity of the mixed solvent decreases the pK value. Thus, an increase in the polarity increases

the solvation of ions and therefore the dissociation. The linear solvation energy relationships obtained (Table 4) permit the pK values of diuretics in any acetonitrile–water mixture up to 70% (w/w) acetonitrile to be known. From a practical point of view, it could be of great interest to apply multiple regression analysis to the whole set of pK values of diuretics and the usual concentration by volume % (v/v), v and weight % (w/w), w, as the intercept variables. In these cases, the third-order polynomials shown in Table 5 are obtained. The equations given in Tables 4 and 5 enable us to know the pK_1 and pK_2 values of the diuretics studied in any binary solvent acetonitrile–water mixture up to 70% (w/w) acetonitrile, and thus permit the interpretation of their acid–base behaviour in these widely used hydro-organic mixtures.

Table 4
Linear solvation energy relationships for pK_1 and pK_2 values of diuretics

Diuretic	Multiparametric equation	r
Ethacrynic acid	$pK_1 = 36.25 - 5.57\alpha - 37.01\beta - 4.25\pi^*$	0.9998
Furosemide	$pK_1 = 26.15 - 1.93\alpha - 22.11\beta - 5.85\pi^*$	0.9994
	$pK_2 = -9.48 + 22.94\alpha + 32.45\beta - 21.19\pi^*$	0.98
Chlorthiazide	$pK_1 = 28.66 - 3.43\alpha - 25.38\beta - 2.75\pi^*$	0.9999
	$pK_2 = -36.42 + 30.28\alpha + 68.16\beta - 23.25\pi^*$	0.998
Trichlormethiazide	$pK_1 = 5.42 + 7.17\alpha + 13.58\beta - 12.31\pi^*$	0.993
	$pK_2 = -6.65 + 8.12\alpha + 17.35\beta - 13.92\pi^*$	0.995
Triamterene	$pK_1 = 10.83 + 1.26\alpha - 4.16\beta - 3.19\pi^*$	0.990
Amiloride	$pK_1 = 20.48 - 3.65\alpha - 8.71\beta - 2.86\pi^*$	0.9990
	$pK_2 = -46.02 + 35.49\alpha + 86.90\beta - 28.76\pi^*$	0.9993

Table 5
Relationships between pK values of diuretics and weight, w, and volume, v, percentages of acetonitrile

Diuretic		r
Ethacrynic acid	$pK_1 = 3.52 + 1.77\times10^{-2}w + 3.18\times10^{-5}w^2 + 2.57\times10^{-6}w^3$	0.999
	$pK_1 = 3.51 + 1.67\times10^{-2}v - 1.17\times10^{-4}v^2 + 3.81\times10^{-6}v^3$	0.999
Furosemide	$pK_1 = 3.95 + 5.33\times10^{-2}w - 8.96\times10^{-4}w^2 + 9.13\times10^{-6}w^3$	0.995
	$pK_1 = 3.93 + 4.83\times10^{-2}v - 8.33\times10^{-4}v^2 + 8.22\times10^{-6}v^3$	0.996
	$pK_2 = 10.43 - 5.52\times10^{-2}w + 2.26\times10^{-3}w^2 - 1.56\times10^{-5}w^3$	0.992
	$pK_2 = 10.61 - 6.50\times10^{-2}v + 2.05\times10^{-3}v^2 - 1.19\times10^{-5}v^3$	0.993
Chlorthiazide	$pK_1 = 6.72 + 1.89\times10^{-2}w - 2.34\times10^{-4}w^2 + 3.95\times10^{-6}w^3$	0.997
	$pK_1 = 6.72 + 1.82\times10^{-2}v - 3.04\times10^{-4}v^2 + 4.32\times10^{-6}v^3$	0.998
	$pK_2 = 9.60 - 2.20\times10^{-2}w + 1.53\times10^{-3}w^2 - 1.23\times10^{-5}w^3$	0.976
	$pK_2 = 9.58 - 1.57\times10^{-2}v + 9.76\times10^{-4}v^2 - 6.18\times10^{-6}v^3$	0.973
Trichlorthiazide	$pK_1 = 7.28 - 1.09\times10^{-2}w + 1.39\times10^{-3}w^2 - 1.13\times10^{-5}w^3$	0.999
	$pK_1 = 7.38 - 0.02v + 1.29\times10^{-3}v^2 - 8.63\times10^{-6}v^3$	0.999
	$pK_2 = 9.90 - 3.44\times10^{-3}w + 1.34\times10^{-3}w^2 - 1.10\times10^{-5}w^3$	0.995
	$pK_2 = 10.05 - 2.10\times10^{-2}v + 1.42\times10^{-3}v^2 - 9.65\times10^{-6}v^3$	0.995
Triamterene	$pK_1 = 6.20 - 1.41\times10^{-2}w + 6.07\times10^{-4}w^2 - 4.06\times10^{-6}w^3$	0.988
	$pK_1 = 6.20 - 1.18\times10^{-2}v + 4.13\times10^{-4}v^2 - 2.05\times10^{-6}v^3$	0.987
Amiloride	$pK_1 = 8.02 + 3.94\times10^{-2}w - 4.60\times10^{-4}w^2 - 3.33\times10^{-6}w^3$	0.999
	$pK_1 = 7.98 + 3.55\times10^{-2}v - 4.01\times10^{-4}v^2 + 2.97\times10^{-6}v^3$	0.999
	$pK_2 = 11.45 - 0.13w + 5.17\times10^{-3}w^2 - 4.25\times10^{-5}w^3$	0.999
	$pK_2 = 11.85 - 0.16v + 4.88\times10^{-3}v^2 - 3.48\times10^{-5}v^3$	0.999

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