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Dissociation constants and preferential solvation of fluoroquinolones in hydroorganic mixtures used in LC

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Abstract

The examination of the protonation equilibria of fluoroquinolones is essential in order to understand their antibacterial activity. The pK values of three representative and widely used fluoroquinolones such as flumequine, norfloxacin and fleroxacin in some acetonitrile–water mixtures were obtained and correlated with the Taft and Kamlet solvatochromic parameters, π^*, α and β. The pK values of antimicrobial quinolones in any acetonitrile–water mixture up to 70% (w/w) can be calculated from the relationships found and the most important solvent properties that affect electrolyte dissociation can also be evaluated. Moreover, variations in the pK values obtained over the whole composition range studied was explained by means of preferential solvation of electrolites in acetonitrile–water mixtures. © 1997 Elsevier Science B.V.

Keywords: Fluoroquinolones; Dissociation constants; Acetonitrile–water mixtures; Preferential solvation; Liquid chromatography

1. Introduction

Many procedures for the separation, detection and quantitative measurement of fluoro-quinolones in biological materials are based on high-performance liquid chromatography methodologies, LC (Degroodt et al., 1994; Mizuno et al., 1994; Barbosa et al., 1996a).

Optimizing the separation is an important part of any methods development project involving LC. In this technique, hydroorganic mobile phases are used and, obviously, the understanding and prediction of retention in LC are of fundamental importance to chromatographers. Most researchers have focused attention on mobile phase optimization, since this is the easiest way to control retention and selectivity in LC. Recently, the linear solvation energy relationship (LSER) based on the Kamlet–Taft multiparameter scales (Park et al., 1988; Carr et al., 1986) was used to

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predict the retention of series of quinolones (Barbosa et al., 1996a), peptides (Barbosa et al., 1996b) and anabolic steroids (Barrón et al., 1995, 1996) in LC. However, this approach allows only for the prediction of retention in mobile solvent mixtures of different compositions, but provides no information about the pH of the mobile phase, which is important in understanding the retention process (Schoenmakers et al., 1991).

It must be emphasized that the interpretation of the ionization effect in non-aqueous mobile phases depends on the pH measurements in hydroorganic solvents and also on pK values of the analytes in these media. Studies of pH-changes aimed at interpreting the ionization effect in non-aqueous mobile phases in LC have been restricted by the limited validity of pH-measurements made with conventional electrodes calibrated in aqueous solutions (Mussini and Mazza, 1987). Moreover, the lack of pK values in hydroorganic mixtures, especially in acetonitrile–water, the mixture most widely used, can involves errors in prediction of the pH required to maximize the retention of solutes by ion suppression.

The standardization of pH measurements in acetonitrile–water mixtures has already been satisfactory performed. In a previous work (Barbosa and Sanz-Nebot, 1995) reference pH-values were assigned to primary standard buffer solutions in order to standardise potentiometric sensors in acetonitrile–water mixtures, according to the NIST multiprimary standard scale (Bates, 1981). So, pH measurements in these media can be performed in a similar way to how they are performed in water (Rondinini et al., 1987).

For pH measurements in acetonitrile–water mixtures, the use of the NIST standard scale with multiple-point calibration procedure (Baucke et al., 1993) is recommended. Multiple-point calibration with linear regression yields high-precision pH values with maximum possible thermodynamic meaning if applied on the basis of the multiprimary standard scale according to NIST (Baucke et al., 1993). Linear regression of several, instead of two, E emf(s)/pH(s) standard couples eliminates both the problem of overdefinition caused by the NIST scale and the bracketing procedure in which the unknown pH(X) is bracketed by a higher and lower standard pH(s) value according to IUPAC (Covington et al., 1985).

On the other hand, a knowledge of acid–base dissociation constants of the analytes in acetonitrile–water which are usually used as mobile phases, can help to improve the analytical methodology and can lead to a better understanding of their biochemical solution. Although only few pK values can be obtained from the literature on acetonitrile–water mixtures (Barbosa et al., 1994, 1997; Mussini et al., 1985a; Pillai et al., 1979), these values imply some important pK differences from the values observed in water. Thus, the study of acid–base behaviour of analytes in acetonitrile–water mixtures could be key in predicting the influence of pH on retention and selectivity in LC.

Fluoroquinolone antibiotics are synthetic, orally active, broad spectrum agents, effective against resistant mutants of bacteria (Hirai et al., 1987; Lockley et al., 1984). They all possess a carbonyl group in position 4 and 6-fluoro and 7-piperazinyl groups that increases their antibacterial activity (Desplaces et al., 1986). The fluoroquinolones are active against many gram-positive and gram-negative bacteria (Joos et al., 1985). The antibacterial activity of fluoroquinolones is pH-dependent since they act by inhibition of bacterial DNA gyrase, a process which depends upon both the pH and concentration of the acid (Neuman, 1988). Therefore, the examination of protonation equilibria of fluoroquinolones is essential in order to understand their antibacterial activity.

The purpose of the present study is the determination of the pK values of three representative and widely used fluoroquinolones, flumequine, norfloxacin and fleroxacin, in 0, 5.5, 10, 16.3, 25, 30, 40, 50 and 70% (w/w) acetonitrile–water mixtures according to the rules and procedures endorsed by IUPAC (Rondinini et al., 1987). The variation of the pK values obtained over the whole composition range studied can be explained by consideration of the preferential solvation of electrolytes in acetonitrile–water

mixtures. Also, in order to obtain pK values in any of the unlimited number of the possible binary solvent acetonitrile–water mixtures, the relationships between pK values and different bulk properties (such as the dielectric constant) were examined and the linear solvation energy relationships method, LSER, applied, in order to study the correlation of pK values with the solvatochromic parameters of acetonitrile–water mixtures. The equations obtained enabled the pK values of the antimicrobials quinolones in any acetonitrile–water mixtures up to 70%(w/w) to be calculated, and thus the acid–base behaviour of these important antimicrobials in widely used acetonitrile–water media to be found.

2. Experimental

2.1. Apparatus

Values of the emf of the potentiometric cell were measured with a Crison 2002 potentiometer (± 0.1 mV) using a Radiometer G 202 C glass electrode and a reference Ag/AgCl electrode prepared according to the electrolytic method (Ives and Janz, 1961) and directly immersed in the solution, to avoid the residual liquid junction potentials (Barbosa and Sanz-Nebot, 1991).

The glass electrode was stored in water when not in use and soaked for 15–20 min in acetonitrile–water mixture before potentiometric measurements. The stabilization criterion for the emf readings was 0.2 mV within 150 s; in all instances the electrode system gave stable and reproducible potentials within 5 min.

The reference electrode was stable for 3 months of continuous work. The E° values used here are the average of at least 15 standardizations. The standardization of the electrode system was carried out each time solvent media or electrodes were changed and the constancy of E° values was ensured by continual surveillance by means of periodical calibrations. The cell was thermostatted externally at 25 ± 0.1°C. The potentiometric assembly was automatically controlled with a microcomputer.

2.2. Reagents

Analytical reagent grade chemicals were used, unless otherwise indicated.

All the solutions were prepared by mixing doubly distilled, freshly boiled water, the conductivity of which did not exceed 0.05 $\mu S \cdot cm^{-1}$, and acetonitrile (Merck, chromatography grade). The quinolones used in this study were purchased from different pharmaceutical laboratories: norfloxacin (Liade, Boral Química), flumequine (Sigma) and fleroxacin (Roche).

Aproximately 10^{-3} M fluoroquinolone solutions were prepared in 10^{-3}M HCl in order to obtain the fully protonated acid–base species.

Stock 0.1 $mol \cdot l^{-1}$ and 0.02 $mol \cdot l^{-1}$ potassium hydroxide (Carlo Erba) solutions were prepared with an ion-exchange resin (Barbosa and Sanz-Nebot, 1991) to avoid carbonation, and standardized volumetrically against potassium hydrogen phthalate.

Stock 0.1 $mol \cdot l^{-1}$, 0.03 $mol \cdot l^{-1}$ and 0.01 $mol \cdot l^{-1}$ hydrochloric acid (Merck) solutions were prepared and standardized titrimetrically against tris(hydroxymethyl)aminomethan (Merck).

2.3. Procedures

The quinolone pK values were determined potentiometrically by titration of appropriate solutions of quinolones in the acetonitrile–water mixtures studied (which contained a measured excess of HCl solution) using KOH solution in the same solvent as titrant. In this way, the quinolones are fully protonated at the beginning of the titration.

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

$$Pt/Ag/AgCl/HA + A$$

$$+ Cl^- (acetonitrile - water)/GlassElectrode$$

where HA and A are the acid and basic species respectively 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:

$$E = E^0 + g\log(a_{H^+} a_{Cl^-}) \quad (1)$$

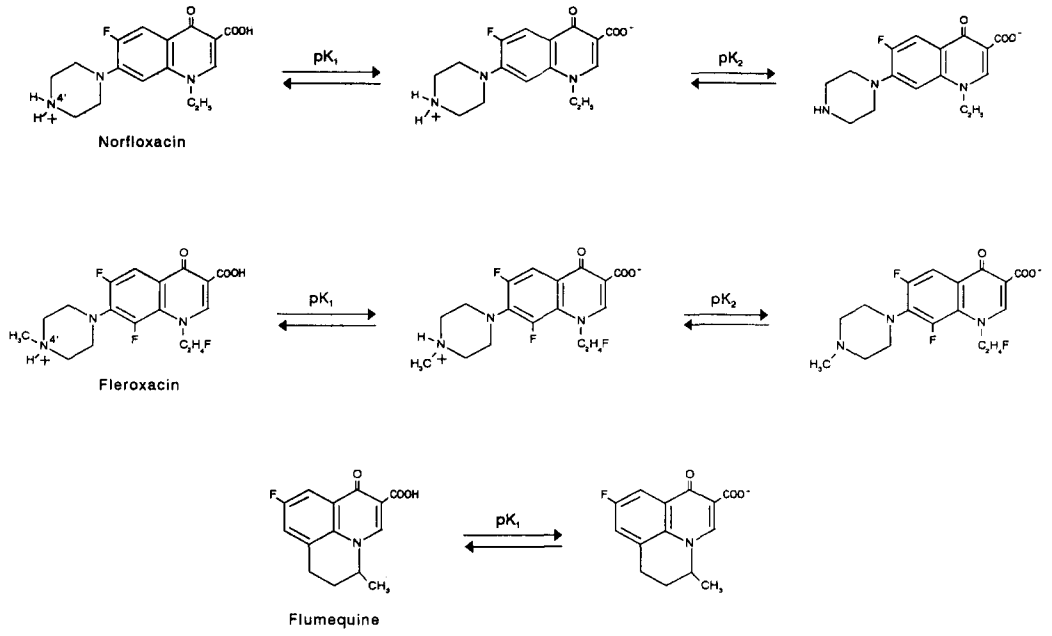

Fig. 1. Structures and protolytic equilibria of selected quinolones.

where E^{θ} is the standard emf of the cell. E^{θ} values were determined as in a previous study (Barbosa and Sanz-Nebot, 1991). Taking into account the general expression for the dissociation equilibria studied

$$K = \frac{c_A y_A c_{H+} y_{H+}}{c_{HA} y_{HA}} \quad (2)$$

the functional Eq. (3), which permits pK calculation, is obtained:

$$\frac{E^o - E}{g} + \log \frac{c_{HA} y_{HA} c_{Cl-} y_{Cl-}}{c_A y_A} = pK \quad (3)$$

where c_{HA} and c_A are the molar concentrations of acidic and basic species, respectively; c_{Cl-} is the molar concentration of the ion chloride; and y_x the molar activity coefficient of species x. The activity coefficients can be calculated through an extrathermodynamic assumption, i.e. a form of the classical Debye–Hückel equation,

$$py = \frac{AI^{1/2}}{(1 + a_o BI^{1/2})} \quad (4)$$

where A and B are the Debye–Hückel constants, a_o is the ion size parameter in the solvent mixture and I is the ionic strength. In compliance with IUPAC rules (Rondinini et al., 1987; Mussini et al., 1985b) the value of the a_oB product in Eq. (4) is assigned at temperature $T = 298.15$ K by an extension of the Bates–Guggenheim convention (Rondinini et al., 1987; Covington et al., 1985), in terms of

$$(a_o B)_T = 1.5(\epsilon^W \rho^S / \epsilon^S \rho^W)_T^{1/2} \quad (5)$$

where ϵ is the dielectric constant, ρ the densities and the superscripts W and S refer to pure water and to the appropriate solvent mixture, respectively. Values of Debye–Hückel parameters A and a_oB at 25°C at different percentages of acetonitrile in admixture with water were reported in previous works (Barbosa and Sanz-Nebot, 1991, 1994a).

Norfloxacin and fleroxacin have two proton-binding sites (Fig. 1). The difference between the two ionization constants is not very high. Therefore, pK calculation was carried out making use of a program written in PASCAL, pKPOT (Bar-

bosa et al., 1995). The least-squares computer program, PKPOT, determines thermodynamic acid–base constants in aqueous and non-aqueous media, taking into account the activity coefficients of the species. These mathematical procedures also determine pK_a values in overlapping ranges ($pK_i - pK_f < 2$) and dissociation constants in very alkaline conditions. The procedures are based on the postulation of a chemical model, that is, the postulation of an initial set of species defined by their stoichiometric coefficients and formation constants, which are then refined in least square minimization.

The statistical analysis of variance was applied to the various independent sets of measurements. The variances that we can hope to estimate are s_w^2, the variance within-sets of data or series of measurements, and s_b^2, the variance between-sets of data. If, after applying the F-test for a 5% level of significance, it is concluded that the two variances do not differ significantly, then, for any given quinolone, all the points of every data set belong to the same population and it should be possible to calculate the total average, pK, and the standard deviation, s, by fitting all the points together and carrying out least-squares analysis. If this hypothesis is rejected, then most of the error derives from the variability between data series, the pK value is obtained by averaging the different intercepts and the total variance $s^2 = s_b^2 + s_w^2$ can be calculated.

In order to study preferential solvation in acetonitrile–water mixtures, pK_a values were obtained from the literature in pure acetonitrile solvent for two acid species of two NIST standard buffers (tartaric and phthalic acids).

3. Results and discussion

The emf, E, of cell A was measured at different concentrations of acidic, HA, and basic, A, species of fluoroquinolones in 0, 5.5, 10, 16.3, 25, 30, 40, 50 and 70% (w/w) acetonitrile–water solvent. 5.5, 16.3 and 25% (w/w) correspond to 7, 20 and 30% (v/v), respectively. For each quinolone various series of measurements were performed, making a total of more than 1800 independent measurements over the solvent interval explored. To simplify the tabulation and as an example, one series of measurements for one titration of fleroxacin and HCl acid solution in 30% (w/w) acetonitrile–water using KOH solution in the same solvent as titrant is shown in Table 1, where V_o is the initial solution volume, V_{ei} the equivalence volumes, c_t the titrant concentration and E^0 the standard emf of cell. For each point of titration, E is the emf value measured, $[H_2A]$ the concentration of fully protonated species, [HA] the concentration of intermediate species, [A] the concentration of basic species and y the molar activity coefficient.

The ionization constant values determined for the involved equilibria for the three fluoroquinolones studied in 0, 5.5, 10, 16.3, 25, 30, 40, 50 and 70% (w/w) acetonitrile–water mixtures are collected in Table 2, together with the respective standard deviations, s, and pK values reported in water (Kitzes-Cohen, 1987; Ross and Riley, 1992, 1994).

Norfloxacin and fleroxacin have two relevant ionizable functional groups (Fig. 1), which means that their acid base chemistry involves two protons, but in contrast, flumequine has only one relevant ionizable functional group within the pH ranges of pharmaceutical or physiological importance. The three quinolones have a carboxyl group that is normally stronger acid than the ammonium group, and it has a pK value of 6 ± 1 in water rich solvents. Therefore pK_1 values can be associated with the carboxylic acid function (Kitzes-Cohen, 1987; Takács-Novák et al., 1990; Ross and Riley, 1992).

The pK_2 values for norfloxacin and fleroxacin can be associated to the presence of a piperazine ring (Fig. 1). Protonation occurs at N_4 of the piperazine ring over other apparently basic sites, as is proven by NMR measurements and by the fact that N_4'-acetylnorfloxacin has only one proton binding group (carboxylate), since the molecule loses amine basicity due to the acetylation of N_4' atom (Takács-Novák et al., 1990).

The pK_1 values associated with the carboxylic acid function for the compounds studied here were higher than those generally observed with carboxylic acids in water mixtures (Barbosa et al.,

Table 1
pK values of fleroxacin in 30% (w/w) acetonitrile–water mixtures

V_o (ml)	V_{e1} (ml)	V_{e2} (ml)	C_t	E°	$[HCl]_o$
20	1.81	3.19	3.98e−2	420.34	3.6e−3

V (ml)	E (mV)	$[H_2A]$	[HA]	[A]	y	$[H^+]$
0.50	−44.02	2.54E−03	1.37E−04	4.61E−07	0.922	4.78E−06
0.60	−68.08	2.34E−03	3.22E−04	2.76E−06	0.923	1.87E−06
0.70	−81.80	2.14E−03	5.03E−04	7.34E−06	0.923	1.10E−06
0.80	−92.03	1.94E−03	6.77E−04	1.46E−05	0.923	7.43E−07
0.90	−100.22	1.76E−03	8.43E−04	2.51E−05	0.923	5.39E−07
1.00	−107.53	1.57E−03	1.00E−03	3.94E−05	0.923	4.07E−07
1.08	−112.76	1.43E−03	1.12E−03	5.41E−05	0.923	3.31E−07
1.17	−118.60	1.27E−03	1.24E−03	7.47E−05	0.923	2.66E−07
1.26	−124.20	1.13E−03	1.35E−03	1.00E−04	0.924	2.16E−07
1.35	−129.70	9.87E−04	1.45E−03	1.32E−04	0.924	1.76E−07
1.44	−135.00	8.55E−04	1.53E−03	1.70E−04	0.924	1.44E−07
1.53	−139.42	7.32E−04	1.60E−03	2.17E−04	0.924	1.18E−07
1.60	−143.56	6.43E−04	1.64E−03	2.59E−04	0.924	1.02E−07
1.89	−160.52	3.48E−04	1.66E−03	4.95E−04	0.923	5.41E−08
2.02	−167.80	2.53E−04	1.60E−03	6.35E−04	0.921	4.08E−08
2.15	−175.50	1.79E−04	1.50E−03	7.92E−04	0.919	3.08E−08
2.24	−180.88	1.38E−04	1.42E−03	9.10E−04	0.918	2.53E−08
2.33	−186.30	1.05E−04	1.31E−03	1.03E−03	0.917	2.07E−08
2.42	−191.70	7.85E−05	1.20E−03	1.16E−03	0.915	1.69E−08
2.51	−197.44	5.70E−05	1.08E−03	1.30E−03	0.914	1.36E−08
2.60	−203.30	4.01E−05	9.52E−04	1.43E−03	0.913	1.09E−08
2.70	−210.20	2.57E−05	8.01E−04	1.59E−03	0.912	8.29E−09
2.80	−218.40	1.51E−05	6.44E−04	1.74E−03	0.910	6.08E−09
2.90	−228.40	7.79E−06	4.82E−04	1.90E−03	0.909	4.18E−09
3.00	−241.90	3.10E−06	3.16E−04	2.06E−03	0.908	2.54E−09

$pK_1 = 6.59$ (0.01).
$pK_2 = 7.91$ (0.01).

1994) (e.g. acetic acid in 30% (w/w) of acetonitrile has a $pK = 5.63$). This decrease in acidity can be attributed to an intramolecular H-bond formation with the neighbouring keto function resulting in stabilization of the protonation species (Ross and Riley, 1992). The formation of an intramolecular hydrogen bond is supported by UV and IR spectral data (Jelikic et al., 1992).

Moreover, the pK_2 value of the norfloxacin, a secondary amine type derivative, is greater than the one of the tertiary amine fleroxacin. These findings were consistent with reports in the literature for similar secondary and tertiary amines: piperazine $pK = 9.71$ (Hetzer et al., 1968) and N-methylpiperazine, $pK = 8.98$ (Enea et al., 1972) in water. The more water molecules involved in the hydrate sphere of the protonated amine, the greater is the stabilization. The protonated form of the secondary amine was stabilized by the greater number of water molecules involved in its hydration sphere when compared with the corresponding tertiary amine (Ross and Riley, 1992).

The few literature pK values of quinolones correspond to their values in water. However, all methods described in the literature for the determination of quinolones by HPLC use acetonitrile–water mixtures as mobile phase (Nilsson-Ehle, 1987; Koechlin et al., 1989; Barbosa et al., 1996a). Thus, although water is not used as mobile phase, the chromatographic behaviour of the quinolones must be explained using their pK values in water. This is a general problem

Table 2
pK values of fluoroquinolones in acetonitrile–water mixtures up to 70% at 298.15 K (values in parentheses are standard deviations)

		Percentage of acetonitrile (w/w)								
		0	5.54	10	16.30	25.03	30	40	50	70
Norfloxacin	pK_1	6.22	—	6.26 (0.05)	6.57 (0.06)	6.81 (0.03)	7.20 (0.04)	7.45 (0.05)	7.98 (0.03)	--
	pK_2	8.38	—	8.48 (0.03)	8.58 (0.01)	8.78 (0.02)	8.72 (0.02)	8.76 (0.05)	9.05 (0.04)	10.01
Fleroxacin	pK_1	5.46	—	5.71 (0.02)	5.93 (0.05)	6.17 (0.04)	6.59 (0.03)	6.60 (0.02)	7.11 (0.03)	—
	pK_2	8.00	—	7.95 (0.06)	8.07 (0.03)	8.05 (0.02)	7.94 (0.05)	8.06 (0.04)	8.39 (0.03)	9.44 (0.04)
Flumequine	pK_1	—	—	6.90 (0.04)	7.09 (0.03)	7.60 (0.03)	7.78 (0.02)	8.11 (0.02)	8.66 (0.02)	9.85 (0.02)

when using HPLC methods. The knowledge of the dissociation constants in hydroorganic media used as mobile phases can be very useful in explaining the chromatographic behaviour of analites. This is one of the reasons why the determination of pK values in hydroorganic media is recommended by IUPAC.

Considering the data shown in Table 2, the extrapolation of pK values in water to acetonitrile–water mixtures is not lineal. This table shows the difficulty of interpreting the variations of pK_1 and pK_2 of fluoroquinolones with the percentage of acetonitrile in the mixtures.

In order to explain that, pK_1 and pK_2 values of fluoroquinolones were plotted against the reciprocal of the dielectric constant (Fig. 2), and against the mole fraction of acetonitrile (Fig. 3). The variation of pK values is different for each substance although, in general, the pK values increase as the acetonitrile content increases. However, pK_1 values corresponding to dissociation of carboxylic acid vary differently from pK_2 ones.

The dissociation constant of a substance HA in a solvent S is related, by means of the Born model, with the dielectric constant of the medium, ϵ, by the following expression (Budewsky, 1979)

$$HA + S \rightleftarrows A^- + HS^+$$

$$pK_a = pK_a^o - pK_{HS^+}^o - e^2(z-1)/(2.3r\epsilon kT) \qquad (6)$$

where K_a^o and $K_{HS^+}^o$ are the intrinsic dissociation constants of the substance and the protonated solvent in the vacuum, taken as standard state, r the average radius of the ions, e the electron charge, z the charge of the acid species HA and kT the energy of thermal agitation.

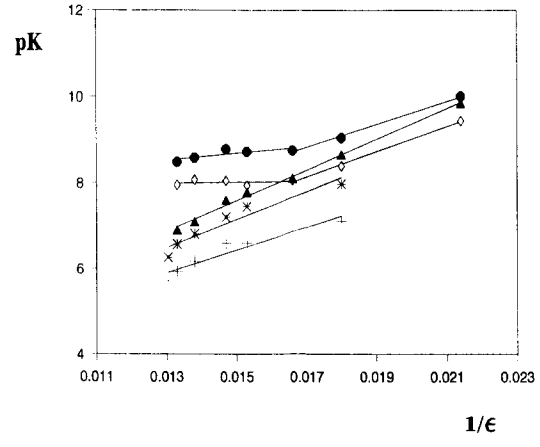

Fig. 2. Plot of pK values of selected quinolones versus the reciprocal of the relative permittivity of solvent mixtures, $1/\epsilon$. (*) pK_1 Norfloxacin; (●) pK_2 norfloxacin; (+) pK_1 fleroxacin; (◇) pK_2 fleroxacin; (▲) pK_1 flumequine.

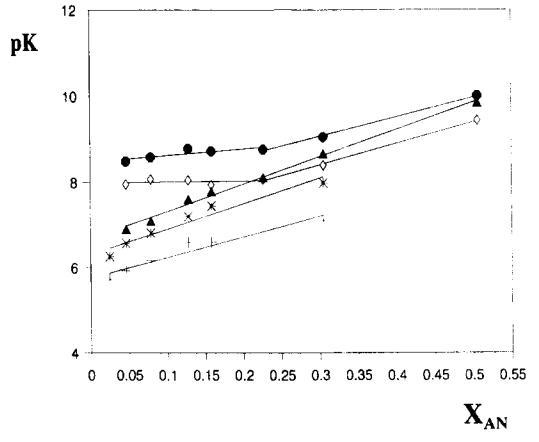

Fig. 3. pK values of selected quinolones as a function of the mole fraction of acetonitrile, x_{AN}. Key to symbols as in Fig. 2.

Table 3
Values of dielectric constants, autoprotolysis constants, and solvatochromic parameters for acetonitrile–water mixtures at 25°C

x_{AN}	wt% AN	ϵ	pK_{ap}	E_T^N	π^*	α	β
0.0000	0	78.36	14.00	1.00	1.14	1.13	0.58
0.0251	5.5	76.68	14.17	0.96	1.12	1.07	0.59
0.0465	10	75.01	14.26	0.93	1.10	1.03	0.59
0.0787	16.3	72.29	14.47	0.90	1.07	0.98	0.60
0.1277	25	68.06	14.74	0.86	1.03	0.94	0.61
0.1583	30	65.52	14.94	0.84	1.01	0.92	0.61
0.2264	40	60.38	15.33	0.81	0.97	0.91	0.61
0.3051	50	55.44	15.73	0.79	0.92	0.90	0.61
0.5059	70	46.82	16.81	0.76	0.84	0.89	0.59

Eq. (6) shows that the medium affects the strength of an acid in two ways: when the acidity of the solvent, $K^{\circ}_{HS^+}$, increases, K_a becomes smaller, and when the dielectric constant decreases, K_a decreases if $z \leq 0$, does not change if $z = 1$ and increases if $z > 1$.

For a series of solvents with similar acidity, the change in the dissociation constant for neutral or anion acids can only be attributed to the change in dielectric constant, and Eq. (6) can be written as:

$$pK_a = A + \frac{B}{\epsilon} \quad (7)$$

where A and B are constants for a given substance.

Thus, the plot of the pK_a of neutral or anion acids vs. the reciprocal of the dielectric constant in some solvents of similar acidity shows a straight line. These predictions are in agreement with plots shown in Fig. 2 corresponding to the dissociation of carboxylic acids of the quinolones, pK_1 values, which were tested experimentally with the ionization constants of pH reference materials in acetonitrile–water mixtures (Barbosa et al., 1994).

However, Eq. (6) and Eq. (7) apply only if all solvents show similar specific solute–solvent interactions, when changes in pK values can be attributed mainly to the change in dielectric constant.

Dissociation of substances in acetonitrile–water is ruled by electrostatic interactions, as well as specific solute–solvent interactions (solvation effects). In the dissociation of neutral or anion acids, charges are created ($HA \rightleftharpoons H^+ + A^-$ or $HA^{n-} \rightleftharpoons H^+ + A^{(n-1)-}$) and the dissociation process is disturbed when the dielectric constant of the medium decreases with the increase in acetonitrile content (Table 3). Hence, for dissociation of the carboxylic acid of the quinolones, pK_1, the electrostatic interaction overwhelm the specific solvation and the pK_1 value increases linearly with the percentage of acetonitrile, as Eq. (7) predicts.

Instead, in dissociation of a monocharged cation acid (such as the ammonium ions of the N_4' of piperazine ring of norfloxacin and fleroxacin, pK_2), there is no change in the number of charges ($HA^+ \rightleftharpoons H^+ + A$) and the change in 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 and the variation with the acetonitrile content is not linear.

The pK_2 values of quinolones in acetonitrile–water mixtures show low changes in the range 0% (w/w) to approximately 30% (w/w) of acetonitrile whereas at higher percentages of acetonitrile they increase with the percentage of acetonitrile (Fig. 2).

The same behaviour is observed for the pK values of fluoroquinolones with the mole fraction of acetonitrile, x_{AN}, as it is shown in Fig. 3. This is of account of in acetonitrile–water mixtures up to 70% (w/w), ϵ^{-1} vs. x_{AN} (the mole fraction of acetonitrile) are related by means of:

$$\epsilon^{-1} = 1.26 \cdot 10^{-2} + 1.73 \cdot 10^{-2} x_{AN} \quad (8)$$

0.9999 being the correlation coefficient of Eq. (8). Thus, Eq. (7) becomes Eq. (9):

$$pK_a = A' + B'x_{AN} \quad (9)$$

As expected, the pK_1 of quinolones increase and pK_2 do not vary in water rich acetonitrile–water mixtures.

The variation of pK_2 values in line with the ammonium ions of quinolones with a percentage of acetonitrile could be explained by the fact that these dissociation constants depend on the solute–solvent interaction effects with the solvents of the mixture. If a solute interacts with one solvent more strongly than with the other, the solute will be preferentially solvated by the former. Preferential solvation by water exists in acetonitrile–water mixtures (Barbosa and Sanz-Nebot, 1994b) and is related to the structural features of these mixtures. In acetonitrile–water mixtures there are three regions (Easteal and Woolf, 1988; Marcus, 1989; Kovacs and Laaksonen, 1991). On the water-rich side there is a region in which the water structure remains more or less intact: the acetonitrile molecules gradually occupy the cavities between water molecules without disrupting the water structure (Easteal and Woolf, 1988). The limit of x_{AN} beyond which the acetonitrile can no longer be accommodated within the cavities of the structure of water is about 0.15 (Marcus and Migron, 1991). In this water-rich region there are not changes in the pK_2 of quinolones. This is in accordance with the previously obtained values of preferential solvation, δ_W, of hydrogen ions by water in acetonitrile–water mixtures (Barbosa and Sanz-Nebot, 1994b). The preferential solvation of hydrogen ions by water is positive, i.e. water molecules show a greater tendency to be in the immediate vicinity of a given hydrogen ion than acetonitrile molecules. In this water-rich region the structure of water remains constant, the solutes are preferentially solvated by water and the variations of pK values are minimal.

In the range $0.15 \leq x_{AN} \leq 0.75$ there are clusters of molecules of the same kind surrounded by regions where molecules of both kinds are near each other. In this middle range of compositions the influence of acetonitrile solvent is high with disruption of water structure. However there is also preferential solvation of hydrogen ions by water (Barbosa and Sanz-Nebot, 1994b), which could explain the low slope of the linear variations of pK_2 values of quinolones vs. x_{AN} plots (Fig. 3). The boundaries of the regions are, of course, not sharp (Marcus and Migron, 1991).

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 and Sanz-Nebot, 1994b).

Although the variation of the pK_1 values of quinolones obtained in acetonitrile–water mixtures with x_{AN} is linear with the mixtures studied, these pK_1 values, as well as pK_2 values, are lower than expected, given the high pK value expected in the neat solvent acetonitrile. Preferential solvation in acetonitrile–water mixtures produce 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 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:

$$pK_s = x_1 pK_{s_1} + x_2 pK_{s_2} \quad (10)$$

where pK_s is the pK value in the mixtures and pK_{s_1} and pK_{s_2} represent the pK values in acetonitrile (solvent 1) and water (solvent 2), 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. pK values of fluoroquinolones in acetonitrile neat solvent are not known but we have obtained from literature the phtalic and tartaric acids in pure solvent (Chantooni and Kolthoff, 1975). The pK values of these acids were determined previously over the whole composition range of acetonitrile–water mixtures (Barbosa et al., 1994). Fig. 4 shows these pK values as a function of x_w, the

bulk mole fraction of water, where the dotted straight line represents the expected variation of pK values between $x_{AN} \approx 0.5$ and pure acetonitrile solvent. Fig. 4 also shows pK_1 and pK_2 values of quinolones vs. x_w for comparison. The pK values obtained could be explained in terms of structural features and preferential solvation by water in acetonitrile–water mixtures. The slope of pK_1 values of quinolones vs. x_w plot is greater than the pK_2 values vs. x_w plot in the water-rich region because of the influence of ϵ.

In water-rich region $x_{AN} \leq 0.15$, pK_2 values do not vary in contrast with pK_1 values because of the influence of changes in ϵ. In the regions where water–acetonitrile mixtures show microheterogeneity $0.15 \leq x_{AN} \leq 0.75$ pK_1 and pK_2 values change but are lower than the theoretical ones because of the preferential solvation by water, and a concave variation of pK vs. x_w may be expected with an inflexion point at $x_w = 0.25$, where preferential solvation by water is maximal (Barbosa and Sanz-Nebot, 1994b).

On the other hand, it is not self-evident that solvatochromic parameters are valid stand-ins for generalized solutes in binary solvent mixtures with regard to the 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 is not solved unequivocally, these investigations provide significant evidence of the general validity of the solvatochromic parameters. 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 the solvent responsible. The Kamlet–Taft (Kamlet et al., 1983) expression states:

$$XYZ = (XYZ)_0 + s\pi^* + a\alpha + b\beta \quad (11)$$

where α, β and π^* are the microscopic parameters previously described, XYZ is the solute property, XYZ_o the value of this property for the same solute in a hypothetical solvent for which $\alpha = \beta = \pi^* = 0$ and a, b and c 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 (Park et al., 1990). Values of the Kamlet–Taft solvatochromic parameters π^* (Marcus and Migron, 1991; Cheong and Carr, 1988), α (Marcus and Migron, 1991; Park et al., 1990) and β (Marcus and Migron, 1991; Krygowski et al., 1985) for acetonitrile–water mixtures over the entire range of composition are known. Table 3 gives the relevant solvatochromic parameter values for the mixtures studied.

Several attempts were made to find the best form of the Kamlet–Taft equation to describe the variation of pK_1 and pK_2 values of fluoroquinolones in acetonitrile–water mixtures. Multiple regression analysis was applied to the pK data. All possible combinations of solvatochromic parameters, including Dimroth and Reichardt normalized parameter E_T^N (Table 3) were checked. The best fit was obtained when the three solvatochromic parameters α, β and π^* were used, yielding the general equations in Table 4. The high coefficient in the β terms compared with the α and π^* terms confirms the main dependence of

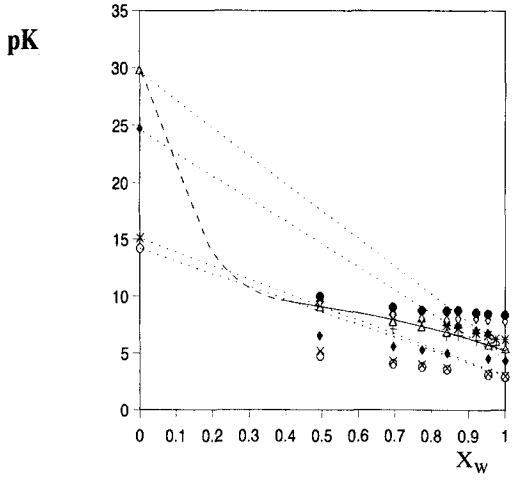

Fig. 4. pK values versus mole fraction of water, x_w, in acetonitrile–water mixtures. (x) pK_1 Tartaric acid; (◆) pK_2 tartaric acid; (○) pK_1 phthalic acid; (△) pK_2 phthalic acid. Remainder of symbols as in Fig. 2.

Table 4
Relationships between pK values of the fluoroquinolones and weight (w), volume (v) percentages of acetonitrile, and linear solvation energy relationships

Substance		
Norfloxacin	$pK_1 = 5.95 + 6.12 \cdot 10^{-2}\, w - 4.12 \cdot 10^{-4}\, w^2$	$r = 0.999$
	$pK_1 = 5.94 + 4.93 \cdot 10^{-2}\, v - 2.28 \cdot 10^{-4}\, v^2$	$r = 0.999$
	$pK_1 = 8.82 - 6.21\pi^* - 1.60\alpha + 10.41\beta$	$r = 0.998$
	$pK_2 = 8.66 - 1.33 \cdot 10^{-2}\, w + 4.56 \cdot 10^{-4}\, w^2$	$r = 0.982$
	$pK_2 = 8.74 - 1.80 \cdot 10^{-2}\, v + 4.43 \cdot 10^{-4}\, v^2$	$r = 0.976$
	$pK_2 = 21.91 - 2.67\pi^* - 1.50\alpha - 14.91\beta$	$r = 0.988$
Fleroxacin	$pK_1 = 5.44 + 5.25 \cdot 10^{-2}\, w - 3.83 \cdot 10^{-4}\, w^2$	$r = 0.996$
	$pK_1 = 5.42 + 4.27 \cdot 10^{-2}\, v - 2.33 \cdot 10^{-4}\, v^2$	$r = 0.996$
	$pK_1 = 7.29 - 4.69\pi^* - 1.67\alpha + 9.27\beta$	$r = 0.997$
	$pK_2 = 8.31 - 3.15 \cdot 10^{-2}\, w + 6.77 \cdot 10^{-4}\, w^2$	$r = 0.988$
	$pK_2 = 8.43 - 3.64 \cdot 10^{-2}\, v + 6.42 \cdot 10^{-4}\, v^2$	$r = 0.983$
	$pK_2 = 20.50 - 3.77\pi^* + 1.50\alpha - 16.48\beta$	$r = 0.990$
Flumequine	$pK_1 = 6.57 + 3.22 \cdot 10^{-2}\, w + 2.05 \cdot 10^{-4}\, w^2$	$r = 0.998$
	$pK_1 = 6.48 + 2.67 \cdot 10^{-2}\, v + 2.20 \cdot 10^{-4}\, v^2$	$r = 0.998$
	$pK_1 = 17.94 - 13.32\pi^* + 4.43\alpha - 1.43\beta$	$r = 0.995$

the pK_1 and pK_2 values of quinolones 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. The linear solvation energy relationships obtained (Table 4) permit to know the pK values of quinolones in any acetonitrile–water mixture up to 70% (w/w) acetonitrile. It could be of great practical interest to apply multiple regression analysis to the whole set of pK values of quinolones and the usual concentration by volume % (v/v), v, and weight % (w/w), w, as the intercept variables. In these cases the second order polynomials shown in Table 4 are obtained. The equations in Table 4 enable the pK_1 and pK_2 values of the quinolones studied in any binary solvent acetonitrile–water mixture up to 70% (w/w) acetonitrile to be known, and thus permit the interpretation of their acid–base behaviour in these widely used hydroorganic mixtures.

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