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Acid–Base Behavior of Quinolones in Aqueous Acetonitrile Mixtures

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Quinolones are a family of antibacterial agents that are used extensively in both human and veterinary clinics. Their antibacterial activity is pH-dependent, and therefore an examination of protonation equilibria in quinolone solutions is essential. p*K*-Values of nine quinolone antibacterials in acetonitrile–water mixtures containing 0, 10, 30, 40, 50 and 70%(w/w) acetonitrile were determined according to the rules and procedures endorsed by IUPAC. In order to obtain quinolone p*K*-values in any acetonitrile–water mixture up to 70%(w/w) acetonitrile, relationships between p*K*-values and different bulk properties (such as dielectric constant) and some microscopic parameters (such as solvatochromic parameters α, β and π^*) were established. These relationships and the application of the preferential solvation theory of electrolytes in acetonitrile–water mixtures permit the interpretation of acid–base behaviour of these important antimicrobials in the widely used acetonitrile–water media.

Many biologically active molecules, such as synthetic drugs and natural products, are fully or partially ionized at physiological pH, and it has often been shown that the presence of charged groups is necessary for biological activity and/or solubility.[1] On the other hand, the un-ionized form has a more favorable partition coefficient towards nonaqueous solvents.[2] Knowledge of the dissociation constants of acidic or basic drugs may therefore be essential for practical purposes (dissolution rates, rates of gastrointestinal absorption, imcompatibilities, etc.) and to interpret structure–activity relationships. In order to emulate the biological conditions, hydro-organic mixtures have been found especially suitable because they simultaneously show low polarity and a partially aqueous content, always present in biological systems. Here, acetonitrile–water mixtures were selected because of the different nature of their constituents, which provides solvent mixtures offering a wide diversity of features and behaviour.

The few literature p*K*-values for organic molecules or biologically active substances reported are often conflicting values for the same compound, and an effort to give thermodynamic significance and homogeinety to data collected is lacking.

Quinolones are a family of antibacterial agents extensively used in both human and veterinary clinics. They are bactericides, and act by inhibiting bacterial DNA-gyrase.[3]

In 1962 Lesher *et al.*[4] isolated nalidixic acid (Fig. 1), and 2 years later it was introduced into general practice for the treatment of urinary infections. Nalidixic acid and other quinolones synthesized later constitute the first-generation quinolone series.

In 1973 Gerster synthesized flumequine.[5] For the first time the C-6 position was functionalized with a fluorine atom (Fig. 1), thus constituting the first synthesis of a fluoroquinolone, also named second-generation quinolones. Its antibacterial activity surpassed that of the first-generation quinolones.

In 1980 Koga[6] synthesized norfloxacin, which was the first fluoroquinolone of the third generation. Another structural modification was performed in this synthesis which is fundamental for the antibacterial activity of these drugs: the C-7 position was functionalized with an aliphatic cyclic amine (piperazine, *N*-alkylpiperazine, etc.) (Fig. 1). It was soon realized that these compounds were much more active *in vitro*, and they showed a broader range of antibacterial activity.

The antibacterial activity of quinolones 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.[7] To this effect, the behaviour of quinolones *in vivo* is significantly influenced by their physicochemical properties, in particular their degree of ionization.[8–10] Therefore, the examination of protonation equilibria in quinolone solutions is essential in understanding their antibacterial activity. Furthermore, the equilibrium constants of antibiotics

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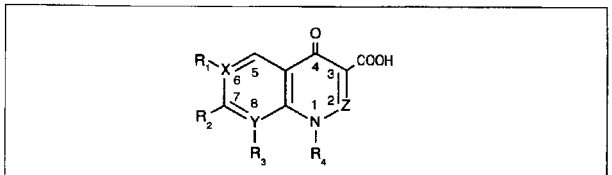


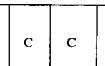
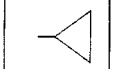
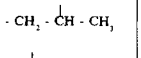
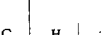
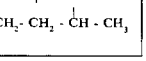
Compound	X	Y	Z	R_1	R_2	R_3	R_4
1.- Ciprofloxacin	C	C	C	F		H	
2.- Norfloxacin	C	C	C	F		H	$-CH_2-CH_3$
3.- Fleroxacin	C	C	C	F		F	$-CH_2-CH_2F$
4.- Ofloxacin	C	C	C	F			
5.- Enoxacin	C	N	C	F		H	$-CH_2-CH_3$
6.- Pipemidic acid	N	N	C	H		H	$-CH_2-CH_3$
7.- Cinoxacin	C	C	N			H	$-CH_2-CH_3$
8.- Nalidixic acid	C	N	C	H	$-CH_2-CH_3$	H	$-CH_2-CH_3$
9.- Flumequine	C	C	C	F	H		

Fig. 1. Structures of selected quinolones.

were biologically important physicochemical parameters. These data are important for a thorough understanding of absorption, transport and receptor binding of these drugs at the molecular level.

On the other hand, reversed-phase liquid chromatography (RPLC) has become an important tool for determination of antimicrobial agents, such as quinolones, in body fluids.

In RPLC, the retention of a weak acid or base is a function of both the ionized and non-ionized species of the compound.[11] For a weak acid, the retention of ionized species (such as A^-) is typically low, whereas the retention of neutral species (such as HA) is much higher. At pH values of the mobile phase adequately lower than pK_a-value of the analite, ionization is supressed and retention is high. At pH values $\gg pK_a$-value, the solute is completely ionized and retention is low. Consequently, the optimization of chromatographic selectivity can be achieved by taking into account the ionization constants of the analites and the capacity factors of the ionized and non-ionized forms.[12] As the chromatographic selectivity is linked to the solute capacity factor ratio, $\alpha = k'_2/k'_1$, the accuracy of pK determination in an aqueous–organic mobile phase plays a major role in the prediction of the optimum location, i.e. of the influence of pH on retention and selectivity in LC.

In recent years we have reported acid–base studies in acetonitrile–water mixtures concerning the standardization of the glass electrode in these mixtures and the determination of their autoprotolysis constant,[13] the determination of ionization constants of pH reference materials in these media,[14] the assignment of reference pH-values to primary standard buffer solutions for standardization of potentiometric sensors in acetonitrile–water mixtures[15,16] and the application of the quasi-lattice quasi-chemical (QLQC) theory[17,18] to the study of the preferential solvation of the hydrogen ions in these mixtures in order to clarify the acid–base behaviour of the solutes in these media.[19]

This work aimes to determine the pK-values of nine quinolone antibacterials in 0, 10, 30, 40, 50 and 70%(w/w) acetonitrile–water mixtures according to the rules and procedures endorsed by IUPAC.[20] The variation of the pK-values obtained over the whole composition range studied can be explained by taking into account the preferential solvation of electrolytes in acetonitrile–water mixtures. Also, in order to obtain pK-values in whichever of the unlimited number of the possible binary solvent acetonitrile–water mixtures, relationships between pK-values and different bulk properties (such as dielectric constant) were examined and relationships with some microscopic parameters, such as solvatochromic parameters α, β and π^*, were obtained. All these equations allow calculation of the pK-values of the quinolone antimicrobials in any acetonitrile–water mixtures up to 70%(w/w) and thus permit the knowledge of the acid–base behaviour of these important antimicrobials in the widely used acetonitrile–water media.

Experimental

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[21] and directly immersed in the solution, to avoid the residual liquid junction potentials.[13]

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 three 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.

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 $\mu S\ cm^{-1}$, and acetonitrile (Merck, chromatography grade). The

quinolones used in this study are shown in Fig. 1, and they were purchased from different pharmaceutical laboratories: pipemidic acid (Almirall, Prodesfarma); nalidixic acid (Impex Química, Sterling Winthrop); norfloxacin (Liade, Boral Química); enoxacin (Almirall); flumequine (Sigma); fleroxacin (Roche); ciprofloxacin (Lasa); cinoxacin (Impex química, Dista); ofloxacin (Hoescht).

Stock 0.1 and 0.01 mol l^{-1} potassium hydroxide (Carlo Erba) solutions were prepared with an ion-exchange resin[13] to avoid carbonation, and standardized volumetrically against potassium hydrogen phthalate.

Aproximately 3×10^{-3} M quinolone solutions were prepared in the solvent mixtures.

Procedures. The quinolone pK-values have been determined potentiometrically by titration of appropriate solutions of quinolones in the acetonitrile–water mixtures studied which also contained approximately 5×10^{-3} M KCl solution for the correct response of electrode system, and using KOH solutions in the same solvent as titrant.

pK-values have been obtained from systematic measurements of the EMF of the cell

$$\mathrm{Pt|Ag|AgCl|HA + A + Cl^-|glass\ electrode}_{\text{in acetonitrile–water}} \qquad (A)$$

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:

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

where E° is the standard EMF of the cell. E°-values were determined as in a previous study.[13] Taken into account the general expression for the dissociation equilibria studied

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

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

$$\frac{E^\circ - E}{g} + \log \frac{c_{HA} y_{HA} c_{Cl^-} y_{Cl^-}}{c_A y_A} = \mathrm{p}K \qquad (3)$$

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

$$py = \frac{AI^{1/2}}{(1 + a_0 BI^{1/2})} \qquad (4)$$

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.

In compiliance with IUPAC rules[20,22] the value of the a_0B product in eqn. (4) is assigned at temperature T= 298.15 K by an extension of the Bates–Guggenheim convention,[20,23] in terms of

$$(a_0 B)_T = 1.5(\varepsilon^W \rho^S / \varepsilon^S \rho^W)_T^{1/2} \qquad (5)$$

where ε is the dielectric constant, ρ the densities, and the superscripts W and S refer to pure water and to the appropiate solvent mixture, respectively. Values of Debye–Hückel parameters A and a_0B at 25 °C at different percentages of acetonitrile in admixture with water are reported in previous studies.[13,24]

In order to check the validity of the results obtained, pK-values were also calculated making use of a program written in PASCAL, pKPOT.[25] The least-squares computer program pKPOT allows the determination of thermodynamic acid–base constants, in aqueous and non-aqueous media, taking into account the activity coefficients of the species. The program also permits the determination of pK_a-values in overlaping ranges ($\mathrm{p}K_i$–$\mathrm{p}K_f < 2$) and dissociation constants in very alkaline conditions. The mathematical procedures are based on the postulation of a chemical model, i.e. the postulation of an initial set of species defined by their stoichiometric coefficients and formation constants, which are then refined in the least-squares minimization. pK-values obtained from eqn. (3) are in accordance with those obtained from pKPOT program.

In order to study preferential solvation in acetonitrile–water mixtures, pK-values for three acid species of three NIST standard buffers (acetic, tartaric and phthalic acids) were obtained from the literature.[26]

Results and discussion

Different EMF measurement series of cell A and pK calculations from eqn. (3) at various concentrations of acidic, HA, and basic, A, species were made for each quinolone studied in 0, 10, 30, 40, 50 and 70%(w/w) acetonitrile–water solvents. For each quinolone in each solvent mixture studied, from three to five series of measurements were performed, for a total of more than 3200 independent measurements over the solvent interval explored. To simplify the tabulation and as an example, one series of measurements for one titration of nalidixic acid in 50%(w/w) acetonitrile–water using KOH solution in the same solvent as titrant is given in Table 1, where V_0 is the initial solution volume, V_e the equivalence volumes, c_t the titrant concentration, [KCl] the initial KCl concentration and E° the standard EMF of the cell. For each point of titration, V is the added volume in mol, E is the EMF measured in mV, [HA] the concentration of acidic species in mol l^{-1}, [A] the concentration of basic species in mol l^{-1} and y the molar activity coefficient.

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 by applying the F-test

Table 1. pK_1-value of nalidixic acid in 50%(w/w) acetonitrile–water mixtures.

V_o	V_e	C_t	[KCl]	E^o		
20	0.90	0.0403	8.91×10^{-3}	449.73		
V	E	[HA]	$[A^-]$	y	pH	pK_a
0.05	−93.5	1.71×10^{-3}	1.01×10^{-4}	0.858	7.00	8.36
0.10	−110.7	1.60×10^{-3}	2.01×10^{-4}	0.858	7.29	8.32
0.15	−122.2	1.50×10^{-3}	3.00×10^{-4}	0.858	7.48	8.31
0.20	−131.7	1.40×10^{-3}	3.99×10^{-4}	0.857	7.64	8.32
0.25	−139.2	1.29×10^{-3}	4.98×10^{-4}	0.857	7.76	8.31
0.30	−146.2	1.19×10^{-3}	5.96×10^{-4}	0.856	7.88	8.32
0.35	−152.5	1.09×10^{-3}	6.93×10^{-4}	0.856	7.99	8.32
0.40	−158.8	9.88×10^{-4}	7.90×10^{-4}	0.855	8.09	8.32
0.45	−164.5	8.87×10^{-4}	8.87×10^{-4}	0.855	8.19	8.32
0.50	−170.4	7.86×10^{-4}	9.83×10^{-4}	0.854	8.28	8.33
0.55	−176.7	6.86×10^{-4}	1.08×10^{-3}	0.854	8.39	8.33
0.60	−183.1	5.87×10^{-4}	1.17×10^{-3}	0.853	8.50	8.33
0.65	−189.9	4.88×10^{-4}	1.27×10^{-3}	0.853	8.61	8.33
0.70	−198.2	3.89×10^{-4}	1.36×10^{-3}	0.853	8.75	8.34
0.75	−207.7	2.91×10^{-4}	1.46×10^{-3}	0.852	8.91	8.35
0.80	−220.5	1.94×10^{-4}	1.55×10^{-3}	0.852	9.12	8.36
0.85	−242.2	9.66×10^{-5}	1.64×10^{-3}	0.851	9.49	8.40
					$pK_a = 8.33$	
					$s = 0.02$	
					$N = 17$	

for a 5% level of significance we can conclude that the two variances do not differ significantly, then, for a given quinolone, all the points of every data set belong to the same population and it should be permissible 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 the 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.

Table 2 shows the ionization constant values determined for the series of nine quinolones studied in 0, 10, 30, 40, 50 and 70%(w/w) acetonitrile–water mixtures and the respective standard deviations, s, together with pK-values reported in water.[27–29]

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.[30–32] 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 using HPLC methods. The knowledge of the dissociation constants in hydro-organic media used as mobile phases can be very useful to explain the chromatographic behaviour of analites. This is one of the reasons why the determination of pK-values in hydro-organic media is recommended by IUPAC.

Quinolone analogues have several potentially ionizable functional groups (Fig. 1). Nalidixic acid, flumequine and cinoxacine have only one relevant ionizable functional group within the pH ranges of pharmaceutical or physiological importance, corresponding to the carboxyl group. In contrast, the other six quinolone derivatives studied (1–6, Fig. 1) have two relevant ionizable functional groups, which means that their acid–base chemistry involves two protons, but only one pH change at the equivalence point can be observed when quinolones are titrated using KOH as titrant. All the quinolones have a carboxyl group that is normally stronger acid than the ammonium group, and have a pK-value of 6 ± 1 in water-rich solvents. Therefore pK_1-values can be associated with the carboxylic acid function[10,28] and pK_2 to the protonated amine function. In this way, the protolytic equilibria of the quinolones can be expressed as in Fig. 2.

In aqueous solution, quinolone derivatives exist mainly in zwitterionic forms between pH 3 and pH 11. The same is true for acetonitrile–water mixtures, and for this reason only the second dissociation constant of quinolone derivatives 1–6 can be determinated by potentiometric titration using KOH as titrant.

The pK_1-values associated with the carboxylic acid function for the compounds studied here were higher than what is generally observed with carboxylic acids in water mixtures[14] [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 keto function in position 4, resulting in stabilization of the protonated species.[28] The formation of an intramolecular hydrogen bond is supported by UV and IR spectral data.[33]

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

		Percentage of acetonitrile (w/w)					
		0	10	30	40	50	70
Ofloxacin	pK_2	8.11 (0.02)	8.17 (0.03)	8.35 (0.04)	8.37 (0.05)	8.76 (0.04)	9.87 (0.02)
Pipemidic acid	pK_2		8.43 (0.05)	8.45 (0.04)	8.41 (0.04)	8.65 (0.04)	9.49 (0.03)
Norfloxacin	pK_2	8.38	8.48 (0.03)	8.72 (0.02)	8.76 (0.05)	9.05 (0.04)	10.01 (0.02)
Enoxacin	pK_2	8.50	8.51 (0.02)	8.38 (0.05)	8.61 (0.05)	8.95 (0.04)	9.81 (0.02)
Fleroxacin	pK_2	8.00	7.95 (0.06)	7.94 (0.05)	8.06 (0.04)	8.39 (0.03)	9.44 (0.04)
Ciprofloxacin	pK_2	8.62	8.38 (0.04)	8.41 (0.04)	8.61 (0.04)	8.95 (0.05)	9.84 (0.03)
Nalidixic acid	pK_1	5.95	6.57 (0.04)	7.42 (0.04)	7.76 (0.04)	8.31 (0.02)	9.55 (0.03)
Flumequine	pK_1		6.90 (0.04)	7.78 (0.02)	8.11 (0.02)	8.66 (0.02)	9.85 (0.02)
Cinoxacin	pK_1		5.05 (0.03)	5.87 (0.04)	6.13 (0.04)	6.72 (0.02)	7.85 (0.03)

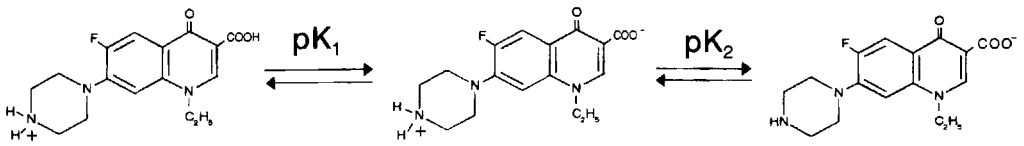


Fig. 2. Protolytic equilibria of norfloxacin.

The pK_2-values of the four secondary amine type derivatives studied (ciprofloxacine, norfloxacine, enoxacine and pipemidic acid) are greater than those of the tertiary amines (ofloxacine and fleroxacine). This is supported by literature data for similar secondary and tertiary amines: piperazine $pK=9.71$[34] and *N*-methylpiperazine, $pK=8.98$[35] in water. The protonated form of the secondary amine was stabilized by the greater number of water molecules involved in its hydration sphere comparing with the corresponding tertiary amine.[28]

Table 2 shows that it is difficult to interpret the variations of pK_1 and pK_2 of quinolones with the percentatge of acetonitrile in the mixtures. Variations are different for each substance although, in general, the p*K*-values increase when the acetonitrile content increases. However, pK_1-values corresponding to dissociation of carboxylic acid vary different than pK_2.

The standard free energy of dissociation of substances in a solvent is made up of two terms: an electrostatic one, which can be estimated by the Born equation, and a non-electrostatic one, which includes specific solute–solvent interactions and solvation phenomena. When the electrostatic effects predominate, then in accordance with the Born model, it is possible to relate the dissociation constant with the dielectric permitivity of the medium, ε, by the following expression[36]

$$pK_a = pK_a^\circ - pK_{HS^+}^\circ - e^2(z-1)/(2.3r\varepsilon KT) \qquad (6)$$

where K_a° and $K_{HS^+}^\circ$ 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.

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

If $z \neq 1$, for a series of solvents with similar acidity (i.e. the different acetonitrile–water mixtures), the variation of dissociation constants with $1/\varepsilon$ should be a straight line:

$$pK_a = A + B/\varepsilon \qquad (7)$$

where A and B are constants for a given substance.

This is the case of flumequine, cinoxacine and nalidixic acid. Correlations between pK_1-values and $1/\varepsilon$ are close to linear, Table 3, with correlation coefficients greater than 0.99.

In the dissociation process of these quinolones (which have only a carboxylic acid site), charges are created ($HA \rightleftharpoons H^+ + A^-$) and the electrostatic interaction overwhelms the specific solvation, and eqns. (6) and (7) are valid.

However, in dissociation of a monocharged cation acid (such as the ammonium ions of the N_4' of piperazine ring of quinolones, pK_2), there is no change in the number of charges ($HA^+ \rightleftharpoons H^+ + A$), and the charge 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.

It can be seen from Table 2 that pK_2-values corresponding to ammonium ion of quinolones in acetonitrile–water mixtures show small changes in the range 0%(w/w) to ca. 30%(w/w) of acetonitrile, whereas at higher percentages of acetonitrile there is a higher variation: p*K*-values increase when the percentage of acetonitrile increases.

This variation could be explained by means of 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[19] and is related to the structural features of these mixtures.[37,38]

In acetonitrile–water mixtures there are three regions: (i) a water-rich region [ca. 0–30%(w/w) acetonitrile] in which the water structure remains more or less intact and the acetonitrile molecules are gradually accommodated within the cavities of waters structure; (ii) an

Table 3. Relationships between pK_1-values of quinolone derivatives and the reciprocal of the dielectric constant and the mole fraction of acetonitrile.

Quinolone				
Nalidixic acid	$pK_1 = 1.75 + 365.1/\varepsilon$	$r=0.999$	$pK_1 = 6.33 + 6.42x_{AN}$	$r=0.999$
Flumequine	$pK_1 = 2.16 + 360.6/\varepsilon$	$r=0.998$	$pK_1 = 6.69 + 6.34x_{AN}$	$r=0.998$
Cinoxacin	$pK_1 = 0.51 + 343.6/\varepsilon$	$r=0.998$	$pK_1 = 4.82 + 6.04x_{AN}$	$r=0.998$

intermediate region [30–85%(w/w) acetonitrile] where water–acetonitrile mixtures show microheterogeneity, i.e. there are clusters of molecules of the same kind surrounded by regions where molecules of both kinds are near each other; in this region, water structure is disrupted by acetonitrile molecules; (iii) an acetonitrile-rich region [>85%(w/w) acetonitrile] with a low number of water clusters and, then, an important decrease of preferential solvation by water.

In the first and second regions [<85%(w/w) acetonitrile] the preferential solvation of protons by water is positive[19] but the structural features are different.

In the water-rich region, the structure of water remains constant, and the variations in pK-values are minimal. In the middle range of compositions the influence of acetonitrile solvent is high with disruption of water structure, and pK-values vary with the percentage of acetonitrile, but preferential solvation of hydrogen ions by water occurs, which could explain the low slope of the linear variations. The boundaries of the regions are, of course, not sharp.[36]

The same behaviour is observed when pK-values are plotted against the mole fraction of acetonitrile, x_{AN}, because ε^{-1} and x_{AN} are related in acetonitrile–water mixtures as shown:

$$\varepsilon^{-1} = 1.26 \times 10^{-2} + 1.73 \times 10^{-2} x_{AN} \qquad r = 0.9999 \qquad (8)$$

As expected, relationships between pK-values of flumequine, cinoxacin and nalidixic acid with mole fraction of acetonitrile are linear, Table 3, and pK_2-values of remaining quinolones (Fig. 1, 1–6) almost change up to 30%(w/w) of acetonitrile, Table 2.

The pK-values of all the quinolones studied are lower than the expected values considering the high pK-value expected in the neat solvent acetonitrile. Preferential solvation in acetonitrile–water mixtures produces lower pK-values than expected if the preferred solvent is water. 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

$$pK_s = x_1 pK_{s_1} + x_2 pK_{s_2} \qquad (9)$$

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.[39] pK-Values of quinolones in acetonitrile neat solvent are not known, but pK-values of phthalic, tartaric and acetic acid have been obtained from the literature.[40] These pK-values were determined previously over the whole composition range of acetonitrile–water mixtures.[14] Figure 3 shows these pK-values as a function of x_w, the bulk mole fraction of water, where the dotted straight lines correspond to the ideal variation of the pK-values and the dashed line represents

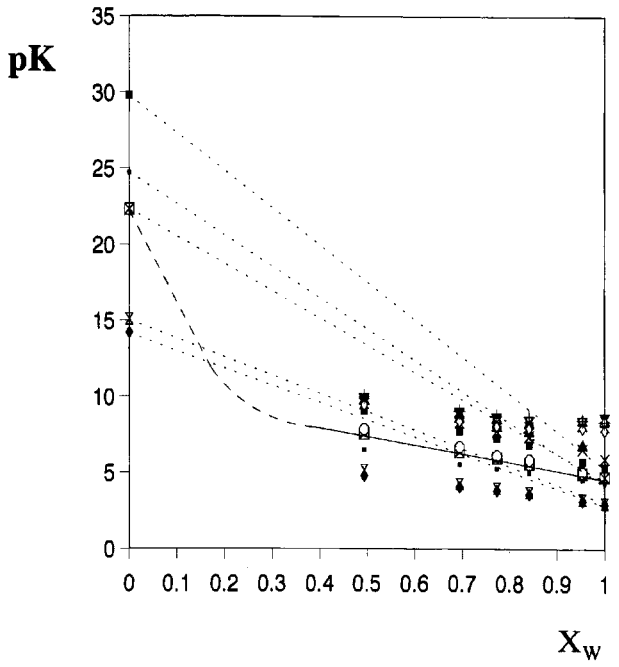


Fig. 3. pK-values of quinolones versus mole fraction of water, x_w, in acetonitrile–water mixtures. *, Ofloxacin; □, Pipemidic acid; +, Norfloxacin; △, Enoxacin; ◇, Fleroxacin; ▼, Ciprofloxacin; ×, Nalidixic acid; ▲, Flumequine; ○, Cinoxacin; ⊠, Acetic acid ; ◆, Phthalic acid, pK_1; ■, Phthalic acid, pK_2; ⧖, Tartaric acid, pK_1; ▪, Tartaric acid, pK_2.

the expected variation of pK-values between $x_{AN} \approx 0.5$ and pure acetonitrile solvent. Owing to the preferential solvation by water, 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.[19] Figure 3 also shows pK-values of quinolones vs. x_w for comparation. The pK-values obtained could be explained in terms of structural features and preferential solvation by water in acetonitrile–water mixtures.

On the other hand, Taft, Kamlet and co-workers proposed the use of solvatochromic parameters in order to evaluate solute–solvent interactions for many Gibbs free energy-related properties including dissociation constants of protonated bases in water,[41,42] through correlation analysis and linear solvation energy relationships (LSER). However, preferential solvation in such mixtures may interfere more seriously with the ability of solvatochromic parameters to act as stand-ins for generalized solutes than in the case of single solvents. Progress has been made,[36,43] and although this problem is not solved unequivocally, these investigations provide significant evidence that the solvatochromic parameters seem to have general validity.

The Kalmet–Taft[41] expression states:

$$XYZ = (XYZ)_0 + s\pi^* + a\alpha + b\beta \qquad (10)$$

where XYZ is the solute property; XYZ_0 is the value of this property for the same solute in a hypothetical solvent for which $\alpha = \beta = \pi^* = 0$; a, b and s are the susceptibilities

Table 4. Linear solvation energy relationships for p*K*-values of quinolones.

Substance		
Ofloxacin	$pK_2 = 23.48 - 3.50\pi^* - 1.17\alpha - 17.23\beta$	$r = 0.990$
Pipemidic acid	$pK_2 = 17.92 - 2.75\pi^* + 1.51\alpha - 13.34\beta$	$r = 0.993$
Norfloxacin	$pK_2 = 20.37 - 4.01\pi^* + 0.51\alpha - 13.30\beta$	$r = 0.996$
Enoxacin	$pK_2 = 13.84 - 7.84\pi^* + 7.38\alpha - 7.11\beta$	$r = 0.999$
Fleroxacin	$pK_2 = 17.86 - 6.04\pi^* + 4.44\alpha - 13.01\beta$	$r = 0.999$
Ciprofloxacin	$pK_2 = 15.74 - 7.15\pi^* + 5.30\alpha - 8.20\beta$	$r = 0.999$
Nalidixic acid	$pK_1 = 22.60 - 10.30\pi^* + 0.59\alpha - 8.77\beta$	$r = 0.999$
Flumequine	$pK_1 = 22.59 - 10.14\pi^* + 0.28\alpha - 7.97\beta$	$r = 0.999$
Cinoxacin	$pK_1 = 19.59 - 10.02\pi^* + 1.00\alpha - 7.50\beta$	$r = 0.998$

Table 5. Relationships between p*K*-values of the quinolones and weight, *w*, and volume, *v*, percentages of acetonitrile.

Substance		
Ofloxacin	$pK_2 = 8.20 - 1.76 \times 10^{-2}v + 5.06 \times 10^{-4}v^2$	$r = 0.985$
	$pK_2 = 8.18 - 1.43 \times 10^{-2}w + 5.41 \times 10^{-4}w^2$	$r = 0.991$
Pipemidic acid	$pK_2 = 8.77 - 3.13 \times 10^{-2}v + 5.28 \times 10^{-4}v^2$	$r = 0.986$
	$pK_2 = 8.68 - 2.75 \times 10^{-2}w + 5.55 \times 10^{-4}w^2$	$r = 0.991$
Norfloxacin	$pK_2 = 8.69 - 2.03 \times 10^{-2}v + 4.87 \times 10^{-4}v^2$	$r = 0.991$
	$pK_2 = 8.61 - 1.44 \times 10^{-2}w + 4.88 \times 10^{-4}w^2$	$r = 0.994$
Enoxacin	$pK_2 = 8.82 - 3.33 \times 10^{-2}v + 6.08 \times 10^{-4}v^2$	$r = 0.999$
	$pK_2 = 8.70 - 2.69 \times 10^{-2}w + 6.15 \times 10^{-4}w^2$	$r = 0.997$
Fleroxacin	$pK_2 = 8.32 - 3.62 \times 10^{-2}w + 6.64 \times 10^{-4}w^2$	$r = 0.996$
	$pK_2 = 8.19 - 2.99 \times 10^{-2}w + 6.81 \times 10^{-4}w^2$	$r = 0.983$
Ciprofloxacin	$pK_2 = 8.62 - 2.58 \times 10^{-2}v + 5.48 \times 10^{-4}v^2$	$r = 0.999$
	$pK_2 = 8.51 - 1.90 \times 10^{-2}w + 5.45 \times 10^{-4}w^2$	$r = 0.999$
Nalidixic acid	$pK_1 = 6.05 + 2.75 \times 10^{-2}v + 2.31 \times 10^{-4}v^2$	$r = 0.997$
	$pK_1 = 6.29 + 2.66 \times 10^{-2}w + 2.82 \times 10^{-4}w^2$	$r = 0.999$
Flumequine	$pK_1 = 6.66 + 1.67 \times 10^{-2}v + 3.27 \times 10^{-4}v^2$	$r = 0.998$
	$pK_1 = 6.60 + 2.97 \times 10^{-2}w + 2.37 \times 10^{-4}w^2$	$r = 0.998$
Cinoxacin	$pK_1 = 4.85 + 1.28 \times 10^{-2}v + 3.45 \times 10^{-4}v^2$	$r = 0.998$
	$pK_1 = 4.79 + 2.52 \times 10^{-2}w + 2.63 \times 10^{-4}w^2$	$r = 0.998$

of the solute property studied to changes in α, β and π*, respectively; and α, β and π* are the microscopic parameters: α measures the solvent hydrogen-bond donor capability, β measures the solvent hydrogen-bond acceptor capability, and π* measures the solvent dipolarity/polarizability. 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.[44] Values of the Kamlet–Taft solvatochromic parameters π*,[36,45] α[36,44] and β[36,46] for acetonitrile–water mixtures over the entire range of composition are known.

Several attempts were made to find the best form of the Kamlet–Taft equation to describe the variation of p*K*-values of quinolones in acetonitrile–water mixtures. Multiple regression analysis was applied to our p*K*-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, yelding the general equations in Table 4. From a practical point of view it could be of great interest to apply multiple regression analysis to the whole set of p*K*-values of quinolones and the usual acetonitrile 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 5 are obtained. The equations given in Tables 4 and 5 enable us to know the p*K*-values of the quinolones 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 hydrorganic mixtures.

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