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Journal of Chromatography A, 919 (2001) 395–406

JOURNAL OF CHROMATOGRAPHY A

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Prediction of electrophoretic behaviour of a series of quinolones in aqueous methanol ☆

D. Barrón, E. Jimenez-Lozano, J. Barbosa*

Department of Analytical Chemistry, University of Barcelona, Martí i Franquès 1–11, 08028 Barcelona, Spain

Received 20 November 2000; received in revised form 20 March 2001; accepted 29 March 2001

Abstract

Quinolones are a family of antibacterial agents used in human and veterinary clinics. The examination of protonation equilibria is essential because their antibacterial activity is pH-dependent. In this work, dissociation constants of quinolones in MeOH–water mixtures were obtained using capillary electrophoresis. The method is based on a model that relates electrophoretic mobility of the solute with pH. The effect of pH, pK_a and activity coefficient on electrophoretic behaviour was considered. Standard pH values for buffer solutions were previously determined in MeOH–water mixtures, and the pH can thus be measured in these media as in water. This model is also used to obtain the optimum conditions for the separation of a series of substances because it allows one to predict the resolution between adjacent peaks from a few experimental data.

Keywords: Buffer composition; Dissociation constants; Electrophoretic mobility; pH effects; Resolution prediction; Quinolones

1. Introduction

Quinolones are chemotherapeutic agents with antibacterial activity and belong to the family of gyrase inhibitors. These compounds are considered the most important group of synthetic antibacterial agents developed since the discovery of sulfonamides and are widely used in human medicine as well as in veterinary medicine. Their use as therapeutic agents in food producing animals in several nations raises serious concerns regarding possible loss of efficacy of these drugs in humans or the development of resistance of human pathogens to antibiotics [1–3].

The analysis of quinolones is dominated by high-performance liquid chromatography (HPLC) and UV detection [4–7]. The application of capillary electrophoresis (CE) techniques for determination of some quinolones has been published recently [8,9]. The use of non-aqueous and hydro–organic media for CE has gained a renewed interest for separation of drug substances due to the high separation selectivity obtained in these systems [10]. A number of solvents, including both protic and aprotic solvents are of interest. Criteria for application of these solvents are their miscibility with water, the ability to dis-

☆ Presented at the 29th Scientific Meeting of the Spanish Group of Chromatography and Related Techniques, Alcalá de Henares (Madrid), 12–14 July 2000.

* Corresponding author. Tel.: +34-93-4021-279; fax: +34-93-4021-233.

E-mail address: barbosa@apolo.qui.ub.es (J. Barbosa).

0021-9673/01/$ – see front matter
PII: S0021-9673(01)00814-7

solve ionic samples and buffers, the transparency to detection light beam, low evaporation properties under experimental conditions and low toxic properties and prices [11–13]. Following these criteria only a small number of solvents remain as potential candidates to be used in CE. The selection of methanol as organic solvent to be added to water was motivated because of their low viscosity which results in rapid separation and high efficiency, their high UV transparency and their high volatility, which make it an appropriate solvent for successful coupling to mass spectrometry. Moreover, the addition of methanol could increase solubility of the drugs in the separation medium [14,15].

The electrophoretic migration of solutes is influenced by the solvent mixtures used due to the change in the size of the solvated ion, the change of the dielectric constant that may influence the equilibrium of the protolytic reaction and the acid–base property of the solute, expressed by its pK_a value, the change due to the differentiating effect of many organic solvents, with this latter effect being the most significant [16]. So, the knowledge of dissociation constants is important in the prediction of migration order of solutes in CE, and moreover in the understanding of certain chemical phenomena such as biological uptake, biological activity, biological transport and receptor binding of these solutes at the molecular level [17].

This paper focuses mainly on the effect of percentage of MeOH on the electrophoretic behaviour of a series of quinolones in several MeOH–water mixtures, up to 30% (w/w) of MeOH, in order to predict the optimum conditions for their separation by CE. The effects of pH, pK_a and activity coefficients were considered, and in consequence pK_a values of these quinolones are determined in the MeOH–water mixtures previously mentioned. The use of MeOH–water mixtures requires the measurement of pH in these hydro–organic media. Standard pH values for buffer solutions in MeOH–water were determined previously [18]; pH can thus be measured in these media as in water. From pH, pK_a, activity coefficients and electrophoretic mobilities, the resolution between pairs of substances was predicted and the optimum conditions for the separation of the quinolones studied were defined.

2. Experimental

2.1. Chemical and reagents

Analytical-reagent grade chemicals were used, unless otherwise indicated. Norfloxacin and flumequine were supplied by Sigma, sarafloxacin and difloxacin by Abbott, ciprofloxacin from LASA, enrofloxacin from Cenavisa, danofloxacin from Pfized, pipemidic acid from Prodesfarma and marbofloxacin from Vetoquinol. The structures of the quinolones are shown in Fig. 1.

Phosphoric acid (85%), potassium hydrogenphthalate, sodium hydroxide and acetone were supplied by Merck (Darmstadt, Germany). Water, with a conductivity lower than 0.05 μS cm^{-1}, was obtained using a Milli-Q water purification system (Millipore, Molsheim, France). MeOH, HPLC grade, was also supplied by Merck.

2.2. Instrumental parameters

All the experiments were performed on a Beckman P/ACE system 5500 (Beckman Instruments, Fullerton, CA, USA). All the CE separations were performed as usual with the cathode at the detector end of the capillary. An uncoated fused-silica CE column 47 cm (40 cm from the inlet to the detector)×75 μm I.D. from Polymicro Technologies (Phoenix, AZ, USA) was used. The temperature of the capillary was kept at 25°C by a liquid coolant in the capillary cartridge. All the injections were performed in the hydrodynamic mode and the quinolones were monitored with a photodiode array detector at 280 nm, except flumequine, which was monitored at 250 nm. The detection was performed at 260 nm when the mixtures of quinolones were injected. The running voltage was 20 kV. Potentiometric measurements were performed with a Crison MicropH 2002 meter (Barcelona, Spain) equipped with a Ross electrode 8102 (Orion Research, Boston, MA, USA).

2.3. Preparation of solutions

The background solvent for the buffer solutions was prepared by mixing the appropriate amounts of

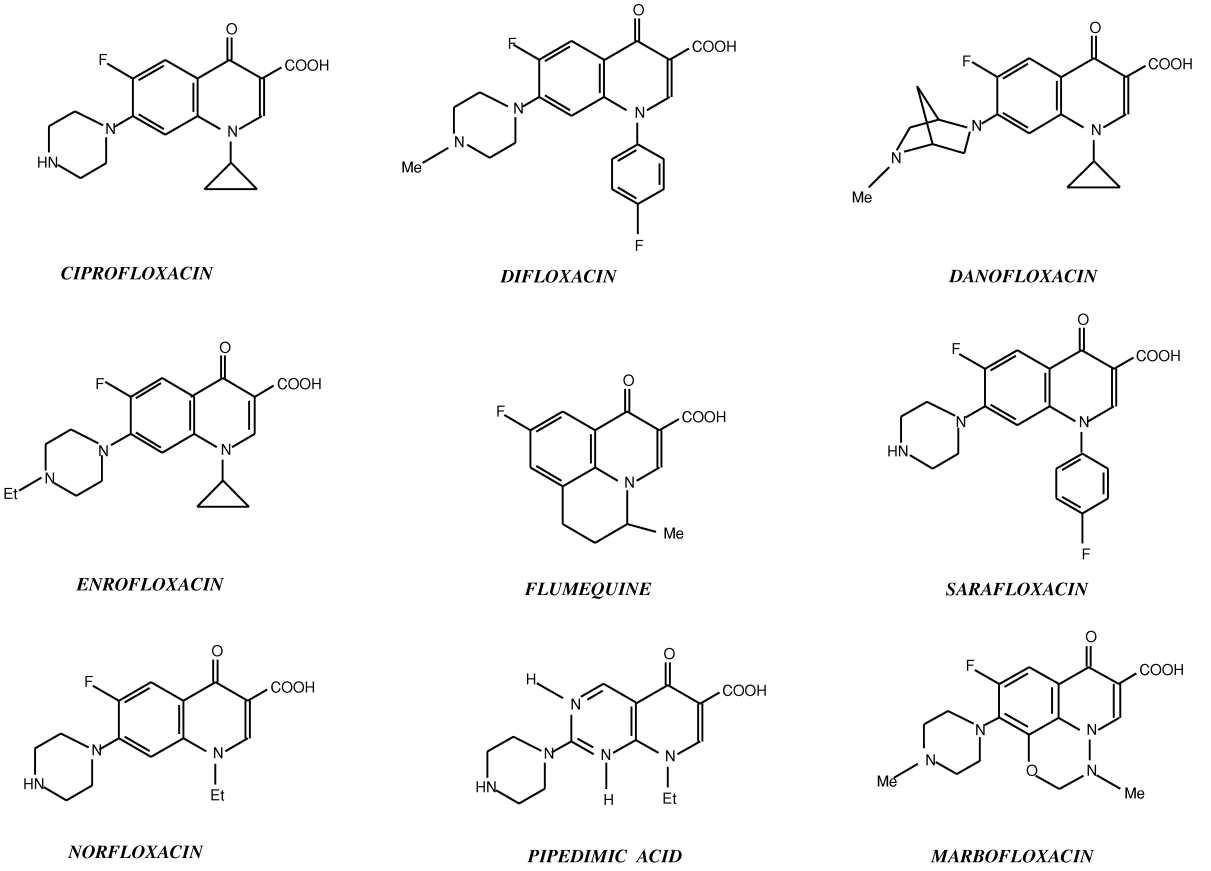

Fig. 1. Structures of quinolones.

water and MeOH to obtain mixtures with 5.5, 10 and 30% (w/w) of MeOH. In order to calibrate the pH meter for pH measurements in the different hydro–organic mixtures, potassium hydrogenphthalate standard buffer (0.1 *M*) was prepared in MeOH–water containing 5.5, 10% and 30% (w/w) of MeOH, with pH values being 4.147, 4.243 and 4.629, respectively [18]. Phosphate working solutions (25 m*M*) were obtained by diluting the concentrated solution of phosphoric acid with the appropriate MeOH–water mixture, and by adding NaOH to adjust the pH.

Working quinolone solutions were prepared in aqueous 50 m*M* acetic acid at a concentration of 25 μg ml^{-1}. The marker of electroosmotic flow used was acetone 3% (v/v), added to each quinolone solution. The solutions used to obtain the mobilities of protonated and anionic species of each quinolone only contained the quinolone studied and the neutral marker. The rest of solutions injected contained all the quinolones studied and the marker. All the solutions were filtered through a nylon membrane with a pore diameter of 0.45 μm.

2.4. Preparation of the capillary

Before changing each buffer, the capillary was purged with 1 *M* sodium hydroxide for 15 min, with Milli-Q water for 20 min and with the appropriate buffer electrolyte for 30 min. Finally, a voltage of 20 kV was applied to the capillary filled with the hydro–organic buffer solution for 20 min. Each day, the system was first purged with 0.1 *M* NaOH for 5 min, then with water for 15 min and finally with the working buffer solution for 20 min. A voltage of

Table 1
Dielectric constants (ϵ), viscosities (η), polarity/polarizability (π^*), densities (ρ), Debye–Hückel parameters (A and a_0B) and autoprotolysis constants (pK_{ap}) at different mass percentages of methanol in water

% (w/w) MeOH	ϵ	100η	ϵ/η	π^*	ρ	A	a_0B	pK_{ap}
0	78.54	0.890	8825	1.17	0.9971	0.5091	1.500	14.00
5.5	76.0	1.02	7451	1.16	0.9885	0.5318	1.518	14.03
10	74.1	1.15	6443	1.16	0.9806	0.5505	1.532	14.08
30	64.3	1.57	4095	1.00	0.9437	0.6695	1.617	14.08

20 kV was also applied for 10 min to the capillary filled with buffer solution. In order to equilibrate the capillary and to minimise hysteresis effects, the capillary was flushed between each run with ultrapure water for 1 min and then with the running buffer for 3 min. Capillaries were stored overnight filled with working buffer electrolyte.

2.5. Procedures

The carrier electrolyte consisted of MeOH–water containing 5.5, 10 and 30% (w/w) of MeOH and phosphate buffer at the appropriate concentration.

The pH of the mobile phase was measured, in accordance with IUPAC rules [19,20] taking into account the reference pH of buffer solutions in MeOH–water mixtures, which were determined in previous work [18]. Thus, the pH_x of a solution was found as follows from:

$$pH_x = pH_s + \frac{(E_s - E_x)}{g} \quad (1)$$

where pH_s and E_s are the pH and electromotive force (e.m.f.) of the standard buffer, pH_x and E_x are the pH and e.f.m. of the carrier electrolyte and g is the Nernst coefficient, $g = (\ln 10)RT/F$.

For each pH assayed, in the range from 2 to 11, working solutions of quinolones (25 μg ml^{-1}) were injected (2 s) in triplicate for several days, in order to obtain constant electrophoretic mobilities. The criterion applied was that the values of electrophoretic mobilities on three consecutive days should differ by less than $2 \cdot 10^{-6}$ cm^2 s^{-1} V^{-1}. Electroosmotic flow was determined from the migration time of acetone, which was considered neutral under experimental conditions [21,22].

In order to determine electrophoretic mobility of the fully protonated and deprotonated species, m_a and m_b, individual solutions of each quinolone and acetone were injected. To determine m_a, these solutions were injected at pH 2 in each MeOH–water mixture studied, that is to say 5.5, 10 and 30% (w/w) of MeOH. For the determination of m_b, the solutions were injected at pH 11 in each MeOH–water mixture. Experimental electrophoretic mobilities were calculated as the difference between the apparent mobility, m_{app}, of each quinolone and the mobility of the neutral marker, m_{EOF} [23].

In order to determine pK_a values and to predict the electrophoretic behaviour of quinolones, the electrophoretic mobility of substances has been related with pH by means of a pK_a dependent equation [24-28], which was obtained assuming that the electrophoretic mobility of a substance is a function of the mobility and the fraction of its species. Most quinolones have a protonated species (H_2Z^+), a zwitterionic species (HZ) and a dissociated species (Z^-). Thus, the electrophoretic mobility, m_e, is given by the equation:

$$m_e = x_{H_2Z^+} m_{H_2Z^+} + x_{HZ} m_{HZ} + x_{Z^-} m_{Z^-} \quad (2)$$

where the term corresponding to the intermediate species is considered nil because the species HZ has no charge and migrates with the electroosmotic flow. Considering $m_{H_2Z^+} = m_a$ and $m_{Z^-} = m_b$, and substituting the terms $x_{H_2Z^+}$ and x_{Z^-} by their expressions for ampholytes: $x_{H_2Z^+} = a_{H^+}^2/(a_{H^+}^2 + K_1 a_{H^+} y + K_1K_2)$ and $x_{Z^-} = K_1K_2/(a_{H^+}^2 + K_1 a_{H^+} y + K_1K_2)$, with y the activity coefficient, the electrophoretic mobility, m_e, can be obtained as follows:

$$m_e = \frac{a_{H^+}^2 m_a - K_1K_2 m_b}{a_{H^+}^2 + K_1 a_{H^+} y + K_1K_2} \quad (3)$$

where m_a, the sign of which being opposite to that of m_b, is the mobility of the fully protonated species, H_2Z^+, and m_b is the mobility of the fully deproto-

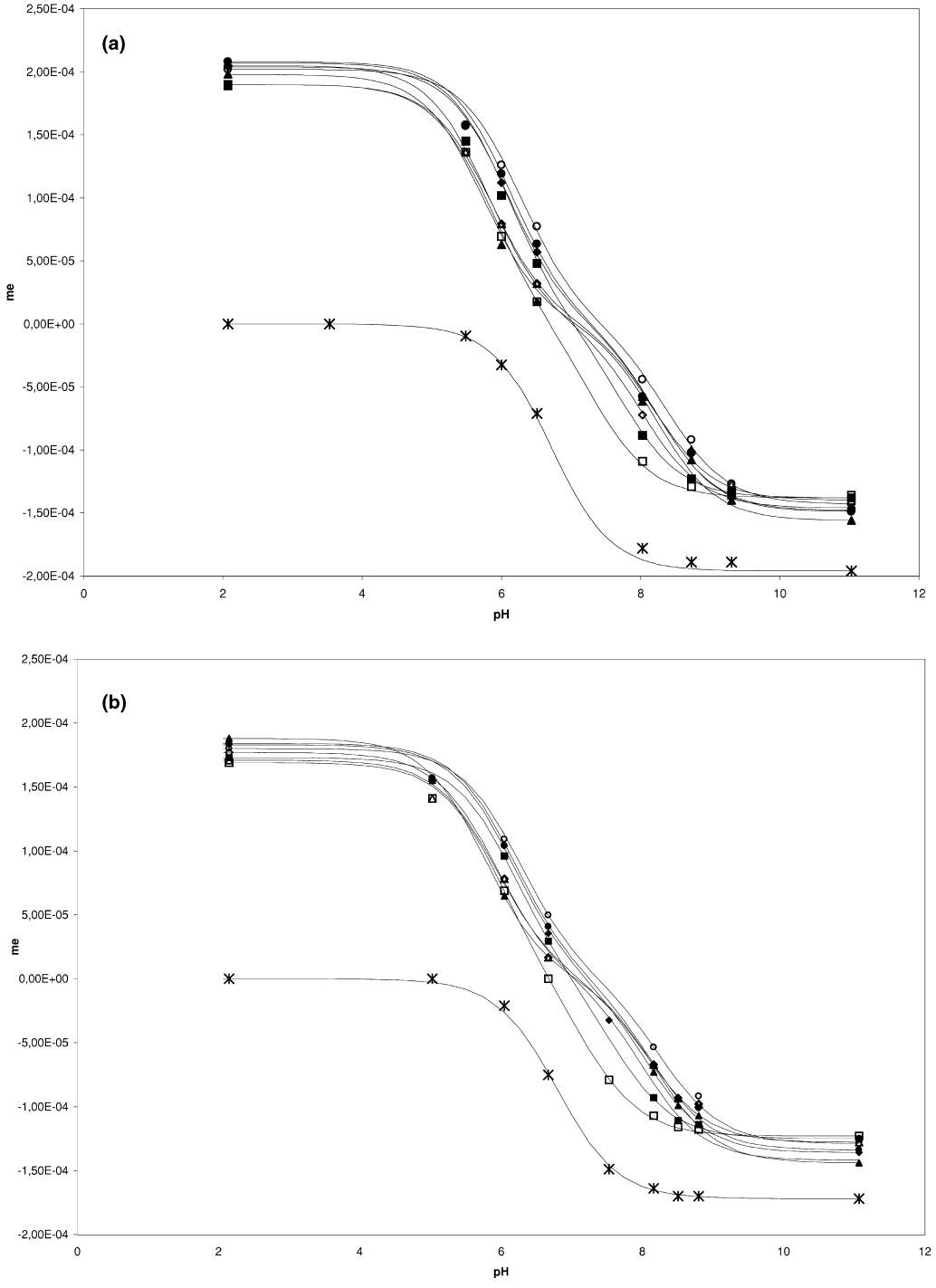

Fig. 2. Plot of electrophoretic mobility of quinolones vs. pH in MeOH–water with (a) 5.5% (w/w) of MeOH, (b) 10% (w/w) of MeOH, (c) 30% (w/w) of MeOH. Symbols: Ciprofloxacin (♦), enrofloxacin (■), pipemidic acid (▲), norfloxacin (●), danofloxacin (○), difloxacin (□), sarafloxacin (△), marbofloxacin (◇), flumequine (*).

nated species, Z^-. Likewise, for acidic substances such as flumequine, we obtain a similar equation:

$$m_e = \frac{K_a m_b}{K_a + a_{H^+} y} \quad (4)$$

where m_b is a negative value. The activity coefficient, y, is obtained, according to IUPAC rules [19,24,25], from the Debye–Hückel equation, $\log y = -AI^{1/2}/(1+a_0BI^{1/2})$, 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 [20] at 25°C at different percentages of MeOH in water are shown in Table 1 for readers convenience.

Eqs. (3) and (4) allow us to determine the acidity constants of analytes when several data pairs of m_e–pH are available. Moreover, they allow us to predict the effect of pH on the electrophoretic mobility of substances when the pK_a of compounds are known, providing the optimum pH for the separation of substances to be predicted.

In order to calculate dissociation constants for each quinolone, data pairs of m_e–pH and ionic strength values in each MeOH–water mixture [mixtures with 5.5, 10 and 30% (w/w) of MeOH] were imported to the NLREG programme [29] where they were fitted to Eqs. (3) or (4), obtaining the dissociation constants values. In this procedure, m_a and m_b were simultaneously refined.

3. Results and discussion

Electrophoretic mobilities obtained for the different mixtures of MeOH–water up to 30% of MeOH, between pH 2 and 11, were plotted against pH of the buffer, and non-linear regression analysis was then performed on the data, using the program NLREG, in order to fit the data to the Eqs. (3) or (4). Fig. 2a shows the best fits of non-linear regression for all the quinolones at MeOH–water with a 5.5% of MeOH; Fig. 2b shows the data obtained at MeOH–water with a 10% of MeOH, while Fig. 2c presents the

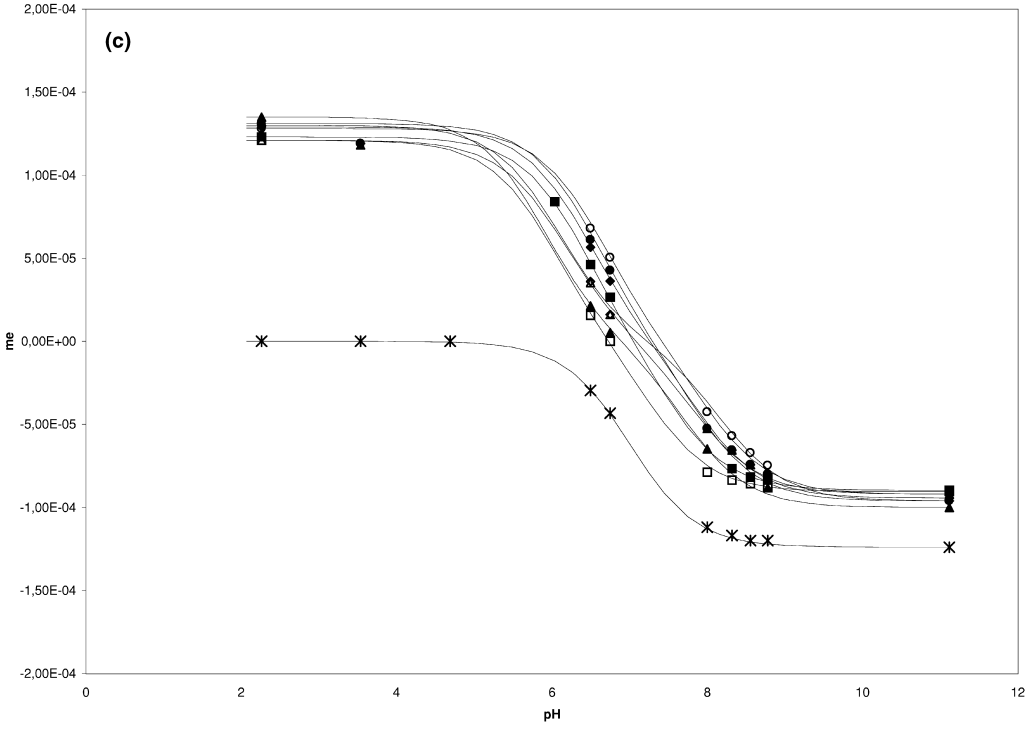

Fig. 2. (*continued*)

electrophoretic mobility obtained in MeOH–water with a 30% of MeOH. The majority of the quinolones in the different mixtures gave two breaks indicating two pK_a values for these substances, except Flumequine that has only one relevant ionisable functional group within the pH ranges of pharmaceutical or physiological importance [30,31] and some quinolones whose pK_a values are too close (differences lower than 1.8) and the two inflexion points merge into one. Data pairs pH–m_e and the ionic strength over the whole pH range, in all the MeOH–water mixtures, were used to determine pK_a values. Initial values for electrophoretic mobility of the fully protonated and deprotonated species, m_a, m_b and pK_1 and pK_2 and activity coefficients are necessary to fit the data to Eqs. (3) or (4). In Table 2, experimental data of m_a and m_b in the different MeOH–water media studied are shown. In Table 2 can be observed that an increase in the percentage of MeOH produces a decrease of the m_a values and a slight rise of the m_b values. The final values for these parameters were obtained from the fit of the data to the equations. The activity coefficients were calculated from the Debye–Hückel equation, whose Debye–Hückel parameters, A and a_0B [20], for all MeOH–water mixtures are shown in Table 1. In this table, values of dielectric constant (ϵ) [32], viscosity (η) [33], polarity/polarizability (π^*) [34], density (ρ) [33] and autoprotolysis constants (pK_{ap}) [35] of MeOH–water mixtures are also included for reader convenience.

The variation in the pK_a values of the quinolones with the solvent composition is presented in Table 3. This variation is different for pK_1 and pK_2 because while pK_1 values increase when the MeOH content increases, the pK_2 values present an opposite behaviour. This behaviour can be explained from the different nature of the equilibria. Dissociation of neutral acids in methanol–water is ruled by electrostatic interactions, as well as specific solute–solvent

Table 2
m_a and m_b values of quinolones obtained in MeOH–water mixtures up to 30% (w/w) of MeOH

Quinolone		% (w/w) MeOH			
		0	5.5	10	30
Ciprofloxacin	m_a	$2.29\cdot10^{-4}$	$2.04\cdot10^{-4}$	$1.83\cdot10^{-4}$	$1.29\cdot10^{-4}$
	m_b	$-1.66\cdot10^{-4}$	$-1.48\cdot10^{-4}$	$-1.36\cdot10^{-4}$	$-9.44\cdot10^{-5}$
Enrofloxacin	m_a	$2.15\cdot10^{-4}$	$1.90\cdot10^{-4}$	$1.73\cdot10^{-4}$	$1.23\cdot10^{-4}$
	m_b	$-1.56\cdot10^{-4}$	$-1.38\cdot10^{-4}$	$-1.25\cdot10^{-4}$	$-8.97\cdot10^{-5}$
Pipemidic acid	m_a	$2.32\cdot10^{-4}$	$1.98\cdot10^{-4}$	$1.88\cdot10^{-4}$	$1.35\cdot10^{-4}$
	m_b	$-1.74\cdot10^{-4}$	$-1.56\cdot10^{-4}$	$-1.44\cdot10^{-4}$	$-1.00\cdot10^{-4}$
Norfloxacin	m_a	$2.29\cdot10^{-4}$	$2.08\cdot10^{-4}$	$1.84\cdot10^{-4}$	$1.31\cdot10^{-4}$
	m_b	$-1.68\cdot10^{-4}$	$-1.49\cdot10^{-4}$	$-1.34\cdot10^{-4}$	$-9.62\cdot10^{-5}$
Danofloxacin	m_a	$2.22\cdot10^{-4}$	$2.02\cdot10^{-4}$	$1.80\cdot10^{-4}$	$1.28\cdot10^{-4}$
	m_b	$-1.59\cdot10^{-4}$	$-1.43\cdot10^{-4}$	$-1.29\cdot10^{-4}$	$-9.21\cdot10^{-5}$
Sarafloxacin	m_a	$2.05\cdot10^{-4}$	$1.90\cdot10^{-4}$	$1.71\cdot10^{-4}$	$1.21\cdot10^{-4}$
	m_b	$-1.57\cdot10^{-4}$	$-1.40\cdot10^{-4}$	$-1.28\cdot10^{-4}$	$-9.21\cdot10^{-5}$
Difloxacin	m_a	$2.02\cdot10^{-4}$	$1.89\cdot10^{-4}$	$1.69\cdot10^{-4}$	$1.21\cdot10^{-4}$
	m_b	$-1.53\cdot10^{-4}$	$-1.36\cdot10^{-4}$	$-1.23\cdot10^{-4}$	$-9.05\cdot10^{-5}$
Marbofloxacin	m_a	$2.16\cdot10^{-4}$	$2.05\cdot10^{-4}$	$1.77\cdot10^{-4}$	$1.30\cdot10^{-4}$
	m_b	$-1.62\cdot10^{-4}$	$-1.46\cdot10^{-4}$	$-1.25\cdot10^{-4}$	$-9.61\cdot10^{-5}$
Flumequine	m_a	0.00	0.00	0.00	0.00
	m_b	$-2.12\cdot10^{-4}$	$-1.96\cdot10^{-4}$	$-1.72\cdot10^{-4}$	$-1.24\cdot10^{-4}$

Table 3
pK_a values of quinolones obtained in MeOH–water mixtures

Quinolone	pK_a	% (w/w) MeOH			
		0	5.5	10	30
Ciprofloxacin	pK_1	5.86 (0.05)	6.02 (0.04)	6.09 (0.06)	6.36 (0.03)
	pK_2	8.24 (0.07)	8.34 (0.06)	8.27 (0.09)	8.07 (0.07)
Enrofloxacin	pK_1	5.88 (0.03)	6.02 (0.03)	6.09 (0.02)	6.30 (0.02)
	pK_2	7.74 (0.03)	7.88 (0.05)	7.76 (0.04)	7.66 (0.05)
Pipemidic acid	pK_1	5.42 (0.03)	5.68 (0.09)	5.70 (0.06)	5.82 (0.09)
	pK_2	8.18 (0.09)	8.34 (0.10)	8.30 (0.07)	7.84 (0.10)
Norfloxacin	pK_1	5.94 (0.05)	6.07 (0.06)	6.12 (0.06)	6.45 (0.05)
	pK_2	8.22 (0.07)	8.36 (0.09)	8.25 (0.09)	8.01 (0.08)
Danofloxacin	pK_1	6.07 (0.06)	6.20 (0.06)	6.21 (0.06)	6.54 (0.04)
	pK_2	8.56 (0.07)	8.50 (0.09)	8.39 (0.08)	8.15 (0.07)
Sarafloxacin	pK_1	5.62 (0.08)	5.80 (0.03)	5.87 (0.07)	6.07 (0.04)
	pK_2	8.18 (0.09)	8.31 (0.05)	8.20 (0.10)	7.98 (0.05)
Difloxacin	pK_1	5.66 (0.04)	5.77 (0.03)	5.88 (0.04)	5.91 (0.10)
	pK_2	7.24 (0.06)	7.48 (0.07)	7.34 (0.05)	7.42 (0.15)
Marbofloxacin	pK_1	5.69 (0.10)	5.74 (0.01)	5.86 (0.04)	6.02 (0.09)
	pK_2	8.02 (0.16)	8.14 (0.02)	8.12 (0.06)	8.30 (0.10)
Flumequine	pK_1	6.61 (0.03)	6.80 (0.02)	6.87 (0.02)	7.10 (0.01)

interactions (solvation effects). In the dissociation of neutral or anionic acids, charges are created ($HA \Leftrightarrow H^+ + A^-$), and the dissociation process is disturbed when the dielectric constant of the medium decreases with the increase in MeOH content. For the dissociation of the carboxylic acid of the quinolones, pK_1, the electrostatic interactions overwhelm the specific solvation and therefore the pK_1 value increase when the MeOH content increases. However, in the dissociation of a cationic acid (such as the ammonium ions of the N_4 of the piperazine ring of quinolones, pK_2) there is no change in the number in the charges ($HA^+ \Leftrightarrow H^+ + A$) and the change in the dielectric constant of the medium does not affect the dissociation process. The dissociation depends only on the solvation of the different species by the solvents of the mixture. In this case, the decrease of the pK_a value by solvation by methanol–water is not balanced by the change on the dielectric constant, and the pK_a value decreases at intermediate methanol compositions. This behaviour has been described previously for cationic acids such as anilinium and ammonium ions [35,36] and has the opposite behaviour that these substances present in other hydro–organic media as acetonitrile–water [37] or tetrahydrofuran–water [38], where pK_2 of quinolones increases with the percentage of organic solvent. Table 3 shows that the variation of pK_1 values of quinolones in the range of MeOH studied are of about 0.5 units. However, in the dissociation of the monocharged cation acid, pK_2 values of quinolones show low changes (with variations of about -0.2 units from 0 to 30% (w/w) of MeOH).

Fig. 2a–c permit the prediction of the effect of pH on electrophoretic behaviour of analytes in the different MeOH–water mixtures in the range of pH between 2 and 11. Based on these figures, the migration behaviour can be determined at any pH,

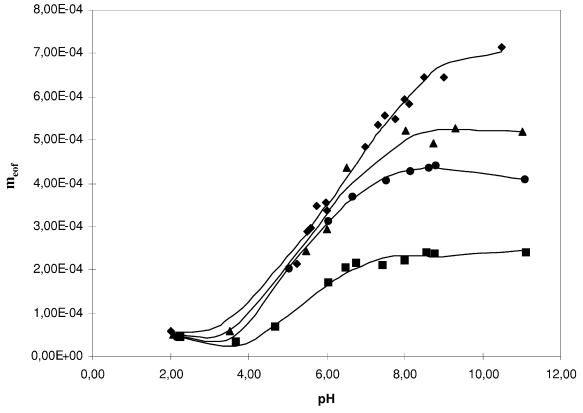

Fig. 3. Dependence of the electroosmotic flow, m_{EOF}, on the pH of the buffer electrolyte in water and in MeOH–water with 5.5%, 10% and 30% (w/w) of MeOH. Symbols: water (■), 5.5% (w/w) MeOH in water (♦), 10% (w/w) MeOH in water (▲), 30% (w/w) MeOH in water (●).

establishing the range of pH in which the mobility values of quinolones are more different and, hence, the pH at which separation is optimum. In these figures, experimental values of m_e are represented by symbols while the better fit of the data to Eqs. (3) or (4) are plotted as curves with solid lines.

From Fig. 2a and b it is deduced that the most suitable separation in the 5.5 and 10% (w/w) of MeOH is around pH 8.5 at which the highest differences between electrophoretic mobilities are found, although the curves predict the comigration of norfloxacin, ciprofloxacin, sarafloxacin and pipemidic acid. However, from Fig. 2c, in the 30% of MeOH, it is deduced that the pH at which the differences between the mobilities of the quinolones are the highest is around pH 6.5, although is expected that sarafloxacin and marbofloxacin migrate together. Fig. 2a–c also permit one to deduce the

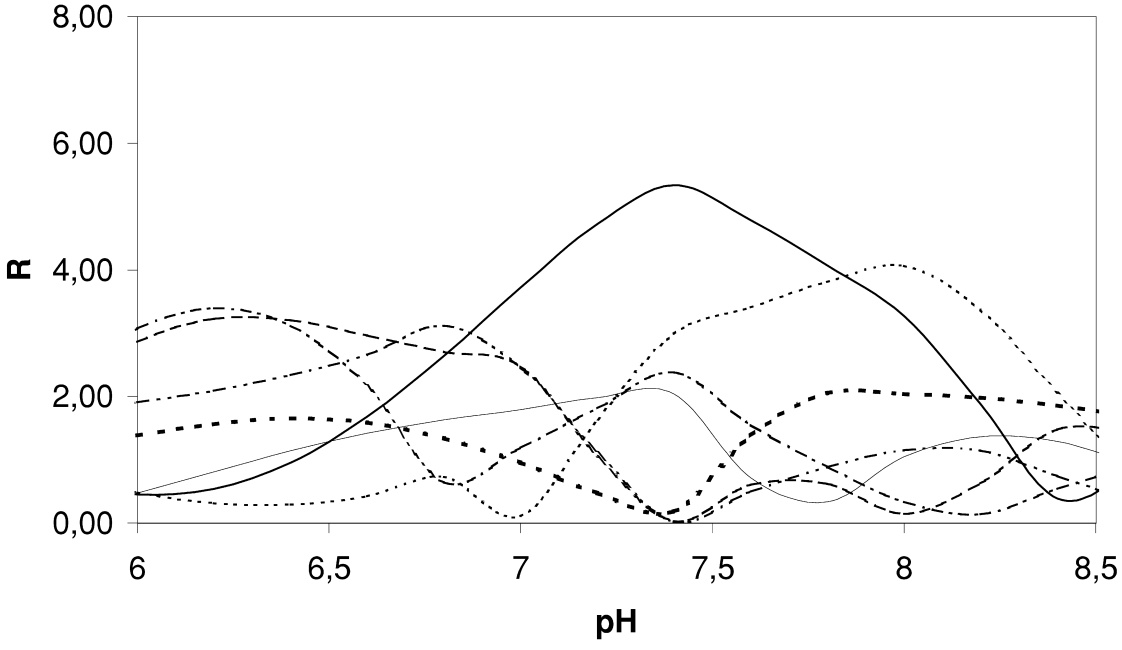

Fig. 4. Predicted resolution for adjacent peaks, obtained from Eq. (5), in MeOH–water mixture with 30% (w/w) of MeOH in the range from pH 6 to 8.5. Symbols: peak 1/peak 2 (—), peak 2/peak 3 (- - -), peak 3/peak 4 (· · ·), peak 4/peak 5 (– · – · –), peak 5/peak 6 (– · · – · · –), peak 6/peak 7 (– – –) peak 7/peak 8 (———).

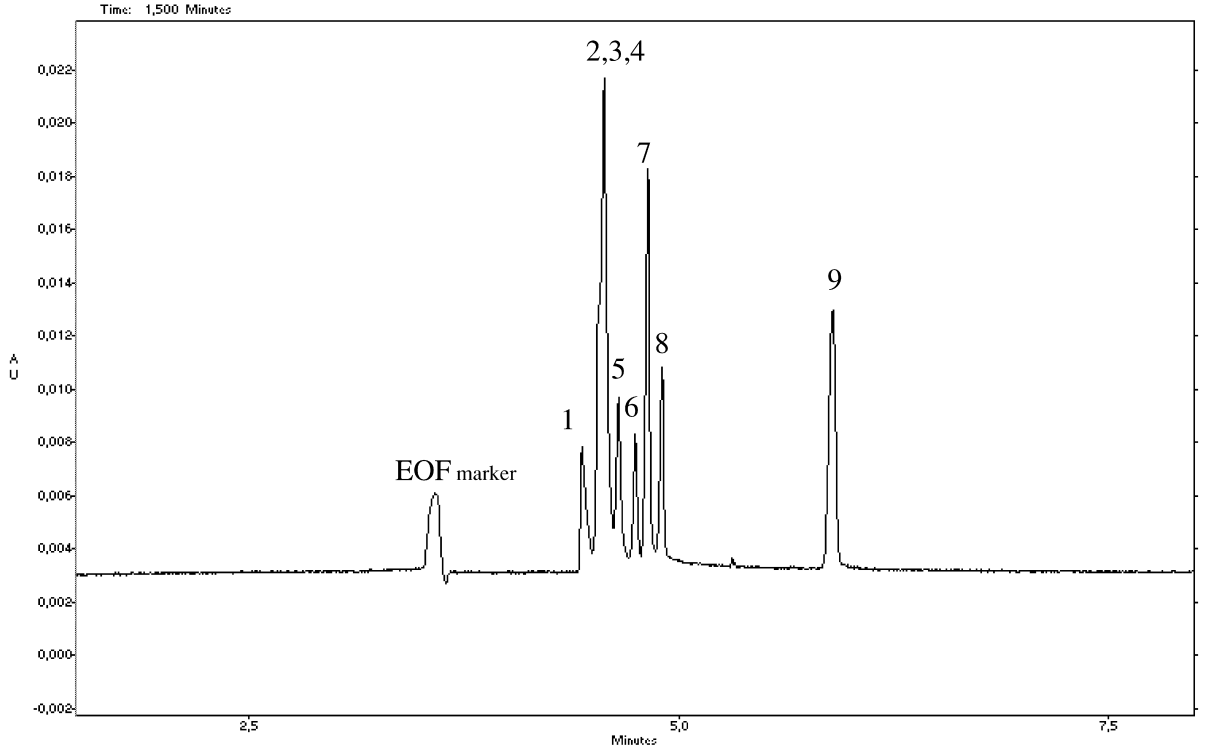

Fig. 5. Electropherogram of a mixture of quinolones using a fused-silica capillary tube 47 cm×75 μm I.D., 25 m*M* phosphate buffer, pH 8.16 in MeOH–water with 10% (w/w) of MeOH, 20 kV, detection wavelength 260 nm. Peaks: (1) danofloxacin, (2) norfloxacin, (3) ciprofloxacin, (4) sarafloxacin, (5) pipemidic acid, (6) marbofloxacin, (7) enrofloxacin, (8) difloxacin, (9) flumequine.

order of migration for substances studied. So, the substances that present major electrophoretic mobility migrate early and so on. In conclusion, the best separation of quinolones studied seems to be achieved with a 30% (w/w) of MeOH at pH between 6 and 7, because only two substances present similar electrophoretic mobilities and in consequence we expect that these substances migrate together.

In order to test these results, theoretical resolution between adjacent peaks was calculated according to the following equation [23]:

$$R = \frac{N^{1/2}}{4} \cdot \frac{(m_2 - m_1)}{m_{\text{avg}} + m_{\text{EOF}}} \quad (5)$$

where m_i is the electrophoretic mobility of the solutes, m_{avg} is the average electrophoretic mobility of the solutes, m_{EOF} is the mobility of the electroosmotic flow and N is the efficiency. Electrophoretic mobility of the solutes are obtained from Eq. (3) for all quinolones except flumequine, for which Eq. (4) was used. m_{EOF} can be evaluated from the study of m_{EOF} vs. pH, Fig. 3, where the electroosmotic mobility of the neutral marker (acetone) is plotted vs. the pH in water and in each of the hydro–organic solvent studied. m_{EOF} was inversely proportional to the concentration of organic solvent. In Table 1 are shown the values of dielectric constant (ϵ) and viscosity (η) and the relation (ϵ/η) for water and MeOH–water with 5.5, 10 and 30% (w/w) of MeOH. As is known, the electroosmotic flow is proportional to the ratio (ϵ/η), and so m_{EOF} decreases when the ratio does, as a result of an increase in the concentration of MeOH. The separation efficiency used to calculate resolution was a minimum value of $5 \cdot 10^4$ theoretical plates. Although higher efficiencies would lead to higher resolution, the ability to predict the optimum pH would not be altered.

Predicted resolution was calculated for quinolones by means Eq. (5), using m_e values predicted from Eqs. (3) and (4) in MeOH–water with 30% of

MeOH. The resolution was calculated considering adjacent peaks at each pH. Fig. 4 shows the predicted resolution for adjacent peaks obtained between pH 6 and 8.5. As can be observed the major number of data presenting resolutions higher than unity is around pH 6.5. In this range, only a predicted resolution has a lower value than unity. The solid line that have this low value of predicted resolution correspond to the pair of peak 3–peak 4 in the range of pH studied. These peaks correspond to sarafloxacin and marbofloxacin. These results are in accordance with the experimental behaviour obtained for the substances studied as can be observed in Figs. 5 and 6. Fig. 5 shows the best separation obtained for the quinolones studied in MeOH–water with 10% of MeOH at around pH 8.2 where norfloxacin, ciprofloxacin and sarafloxacin migrate together. Fig. 6 shows the separation obtained in MeOH–water with 30% of MeOH. This electropherogram was obtained at pH 6.50, and the results agreed with the predictions. The migration order was: danofloxacin, norfloxacin, ciprofloxacin, enrofloxacin, sarafloxacin + marbofloxacin migrating together, pipemidic acid, difloxacin, acetone and flumequine that, at this pH have a negative net charge and was detected after the electroosmotic flow marker.

From the results presented, we can conclude that the model proposed can be suitable to optimise the separation of substances. Our results show that the best separation for quinolones is obtained with MeOH–water with 30% (w/w) of MeOH at pH 6.5, although sarafloxacin and marbofloxacin migrate together.

Acknowledgements

The financial support of DGYCIT of the Spanish Government (project PB98-1174) is gratefully acknowledged. The authors thank Abbott S.A., Cenavisa

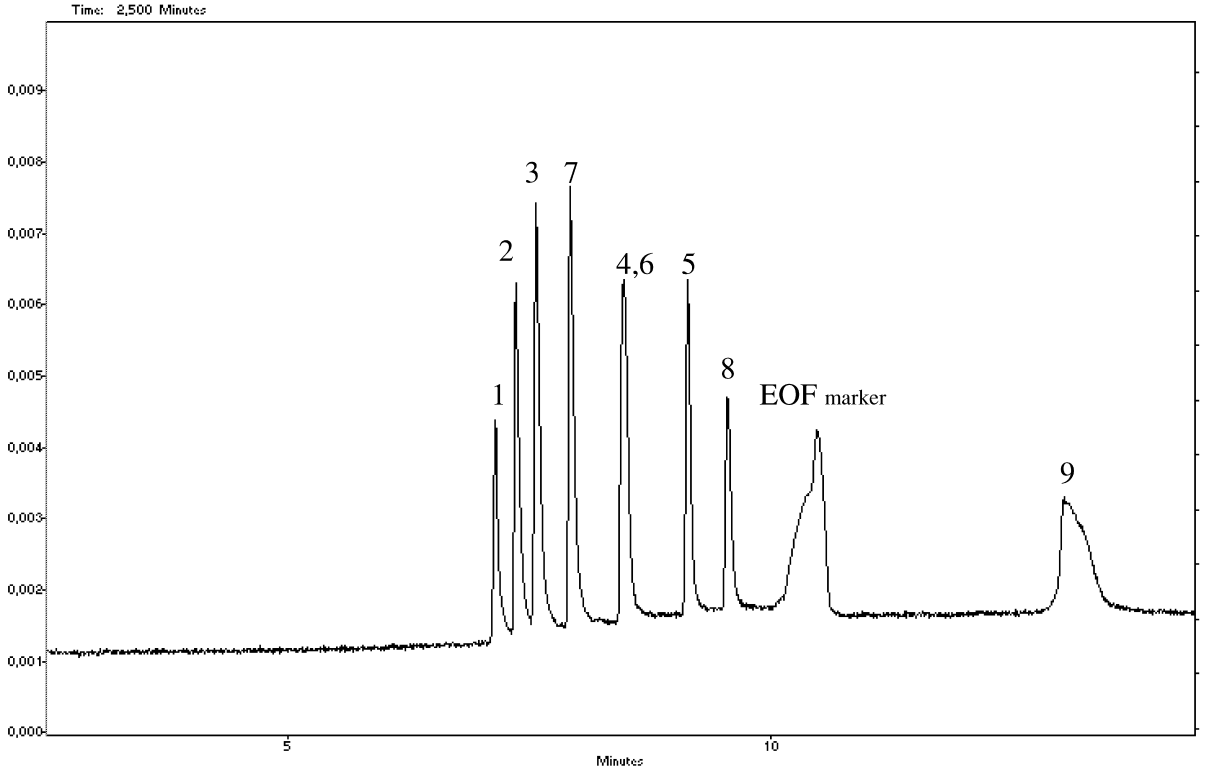

Fig. 6. Electropherogram of a mixture of quinolones using a fused-silica capillary tube 47 cm×75 μm I.D., 25 m*M* phosphate buffer, pH 6.50 in MeOH–water (30:70, w/w), 20 kV, detection wavelength 260 nm. Peaks: (1) danofloxacin, (2) norfloxacin, (3) ciprofloxacin, (4) sarafloxacin, (5) pipemidic acid, (6) marbofloxacin, (7) enrofloxacin, (8) difloxacin, (9) flumequine.

S.A., LASA, Pfized S.A. and Vetoquinol Laboratories for their kind donations of the quinolone standards.

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