ELSEVIER

Journal of Chromatography A, 839 (1999) 183–192

JOURNAL OF CHROMATOGRAPHY A

Electrophoretic behaviour of quinolones in capillary electrophoresis[☆] Effect of pH and evaluation of ionization constants

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

Department of Analytical Chemistry, University of Barcelona, Avda Diagonal 647, 08028 Barcelona, Spain

Received 5 November 1998; received in revised form 22 December 1998; accepted 29 December 1998

Abstract

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 solution is essential. In this work, dissociation constants of quinolones in water were obtained using capillary electrophoresis (CE). The method is based on measuring the electrophoretic mobility of the solute as a function of pH. Mobility and pH data are fitted using different models. These developed equations have two advantages. They permit the determination of pK_a of analytes with the advantages of CE and also permit the prediction of the effect of pH on the electrophoretic behaviour of substances and then the prediction of the pH optimum for the separation methods, using the minimum of experimental measurements.

Keywords: Dissociation constants; Pharmaceutical analysis; Quinolones

1. Introduction

Quinolones exhibit bactericidal activity primarily by inhibiting bacterial DNA gyrase. Fluorinated quinolones differ from their predecessors in their broad antibacterial spectrum, including both Gram-negative and Gram-positive bacteria. They also exhibit high potency, a low incidence of resistance, high oral bioavailability, extensive tissue penetration, low protein binding and long elimination half-lives [1,2]. Owing to their favourable antibacterial and pharmacokinetic profile, the use of quinolones is not limited to clinical applications. These agents are also applied in the treatment and prevention of veterinary diseases in food-producing animals and in commercially farmed fish [1,3]. The wide application range and the extensive use and misuse of quinolones in veterinary medicine represents a potential hazard because residues of these drugs may persist in edible tissues, so the need to identify quinolones in various biological tissues and fluids is obvious. Maximum residue limits (MRLs) of veterinary medicinal products in foodstuffs of animal origin are established at Community level under Council Regulation (ECC), no. 2377/90 [4].

Many biologically active molecules are fully or partially ionized at physiological pH, and it has often

[☆]Presented at the 27th Scientific Meeting of the Group of Chromatography and Related Techniques of the Spanish Royal Society of Chemistry, Lugo, 8–10 July 1998.

*Corresponding author.

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00093-X

been shown that the presence of charged groups is necessary for biological activity and/or solubility. Therefore, the examination of protonation equilibria in drugs solutions is essential in order to understand their activity. The dissociation constants constitute important data for a thorough understanding of absorption, transport and receptor binding of these drugs at the molecular level [5].

The determination of dissociation constants of weakly acidic or basic compounds by established techniques is routine if the compounds have amenable physical properties. However, the low solubility of many pharmaceutical compounds in water and similar absorption properties of species, as is the case of quinolones [6,7] precludes traditional pK determinations.

Capillary electrophoresis (CE) has been introduced as a method for convenient and precise aqueous pK_a determination [8–12] compared with other techniques such as potentiometry, spectroscopy or conductivity. The advantages of using CE to determine accurate thermodynamic pK values of compounds are numerous: CE requires small amounts of sample at low solute concentration, and the procedure does not require measurement of solute or titrant concentrations like potentiometric techniques, but only of migration times. On the other hand, calculations are straightforward and independent of solute purity, since impurities can be separated from the solutes of interest [9,10]. Moreover, CE permits pK_a determination in aqueous solutions without difficulties which is not the case for liquid chromatography, in which the retention could be very important without addition of an organic modifier [13–15].

In this work we investigated the effect of pH on the electrophoretic behaviour in CE of a series of four representative and widely used quinolones by CE. Difloxacin, Flumequine and Danofloxacin were chosen because they are included in Council Regulation 2377/90, and have established MRLs and Norfloxacin was chosen because of its importance and wide therapeutic use. The relationships obtained allow the determination of the pK_a values of substances from few experimental migration time data and, also permit the prediction of the influence of the pH on CE methodologies. Thus, dissociation constants of the substances studied were obtained by using two different models, the direct model [9] and the simultaneous model. The latter was developed in this study in order to obtain pK_a values of zwitterionic substances with very close values. The methods developed in this study are applicable to a wide variety of organic compounds.

2. Experimental

2.1. Chemical and reagents

Norfloxacin and Flumequine were supplied by Sigma; Difloxacin by Abbott and Danofloxacin was from Pfizer. The structures of the quinolones studied are shown in Fig 1. Phosphoric acid (85%), boric acid, sodium hydroxide and acetone were supplied by Merck and acetic acid was obtained from Carlo Erba. All chemicals used in the preparation of buffers and solutions were analytical reagent grade. Water, with a resistivity of 18.2 MΩ cm, was obtained using a Milli-Q water purification system (Millipore, Molsheim, France).

2.2. Preparation of solutions

Several buffer systems covering the pH range between 5.25 and 9.00 were selected. Each buffer was prepared with phosphoric acid and adjusted to the appropriate pH with NaOH. The concentration of the buffer was 0.050 *M* and 0.025 *M* in the acidic and basic media respectively. In order to determine the electrophoretic mobility of the fully protonated quinolone a phosphate buffer of 0.05 *M* at pH 2.00 was used. For the determination of the electrophoretic mobility of the fully deprotonated quinolone two buffers was used: a 0.025 *M* phosphate buffer at pH 11.00, and a 0.025 *M* borate buffer at pH 10.50. Working solutions of each quinolone were prepared at concentrations of 100 ppm and 50 ppm, in 0.05 *M* acetic acid, for use in acidic and basic media respectively. In our work, we have verified the influence of different media, such as aqueous acetic and phosphoric acids, water and acetonitrile, on electrophoretic behaviour of quinolones. In all events no significant differences were observed but better solubility of quinolones were obtained in aqueous acetic acid. Each quinolone solution contained ace-

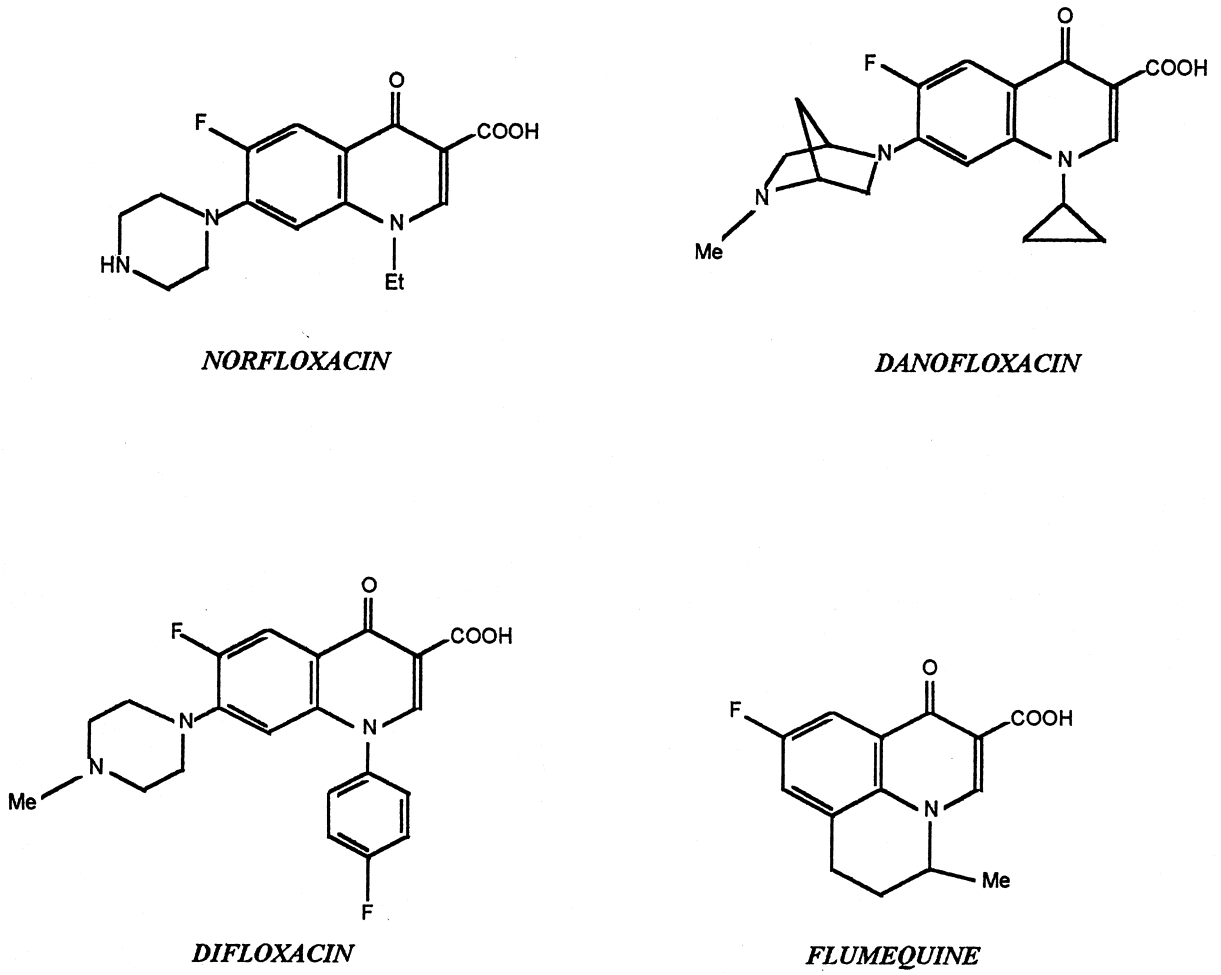

Fig. 1. Structures of selected quinolones.

tone at 3% (v/v) as the electroosmotic flow (EOF) marker [16,17]. All the solutions (quinolones and buffers) were passed through a 0.45-μm filter.

2.3. Instrumental parameters

All CE experiments were performed on a P/ACE System 5500 (Beckman Instruments, Palo Alto, CA, USA) equipped with an autosampler, automatic injector and a photodiode array detector. An untreated fused-silica capillary of 47 cm (40 cm to the detector)×75 μm I.D., (Polymicro Technologies Phoenix, AZ, USA) was used. Samples were injected hydrodynamically at 0.5 p.s.i. for 6 s (1 p.s.i.= 6894.76 Pa). The experiments were performed at 20 kV at 25±0.1°C. The electropherograms were recorder using a computer program (P/ACE Station 1.0 with interface Golden System) supplied by Beckman. Norfloxacin and Danofloxacin were monitored at 280 nm, Difloxacin at 275 nm, and Flumequine was detected at 250 nm. Acetone was also monitored at 280 nm. The pH of the buffer solutions was measured with a Crison 2002 potentiometer (Crison Instruments, Barcelona, Spain) using a Ross electrode 81-02 supplied by Orion Research (Boston, MA, USA).

2.4. Preparation of the capillary

Before each buffer was changed, the capillary was purged with sodium hydroxide 1 *M* for 15 min, followed by Milli-Q water for 20 min and buffer electrolyte for 30 min. The last step was the application of a voltage of 20 kV for 20 min with the capillary filled with buffer solution. Every day the system was first purged with 0.1 *M* NaOH for 5 min followed by water for 15 min and working buffer solution for 20 min. A voltage of 20 kV was also applied for 10 min, with the capillary filled with buffer solution. Between each run the capillary was flushed successively with ultrapure water for 1 min followed by running buffer for 3 min in order to equilibrate the capillary and thereby minimize hysteresis effects. Capillaries were stored overnight filled with working buffer electrolyte.

2.5. Procedures

Standard solutions of each quinolone with acetone were injected in triplicate and were monitored at the corresponding wavelength, previously indicated. To check that electrophoretic mobilities were constant, the injections were repeated over a period of several days. The criterion for constant electrophoretic mobility was that the values of 3 different consecutive days differed in less than $2\cdot10^{-6}$ $cm^2\,s^{-1}\,V^{-1}$. The injections were repeated until this criterion was fulfilled. Electroosmotic flow was determined from the migration time of acetone, which was considered to be neutral throughout the entire sequence of buffers used in the determination of p*K* values [16,17]. Electrophoretic mobilities were calculated as the difference between the apparent mobility, m_{app}, of each quinolone and mobility of the neutral marker, m_{EOF}, [18] using the equation:

$$m_e = m_{app} - m_{EOF} = \frac{L_D L_C}{V}\left(\frac{1}{t_{app}} - \frac{1}{t_{EOF}}\right) \quad (1)$$

where L_D is the distance from the injection point to the detector, L_C is the capillary length, and t_{app} and t_{EOF} are the migration time of the quinolone and the neutral marker respectively.

For Norfloxacin, Danofloxacin and Difloxacin, two acid–base equilibria must be considered [19] (Fig. 2). First [9,10] these two equilibria are considered independently. Thus, the first dissociation of these quinolones is related with the concentration of protonated, $[H_2Z^+]$, and undissociated [HZ] species (Fig. 2), pH and the activity coefficient of the protonated species, $y_{H_2Z^+}$:

$$pK_1 = pH + \log y_{H_2Z^+} - \log \frac{[HZ]}{[H_2Z^+]} \quad (2)$$

This equation is valid assuming that the activity coefficient of the undissociated species, y_{HZ} is 1 [20,21]. The values of y_{HZ} are close to unity and the influence on the definitive value of pK_a is less than their S.D. values.

Taking into account the Debye–Hückel theory, log $y_{H_2Z^+}$can be substituted and Eq. (3) is obtained:

$$pK_1 = pH - \log \frac{[HZ]}{[H_2Z^+]} - \frac{A\sqrt{I}}{1 + a_0 B\sqrt{I}} \quad (3)$$

where *A* and *B* are the Debye–Hückel parameters, a_0 is the hydrated diameter of an ion (Å), and *I* is the ionic strength of the solution. In water, *A* has a value

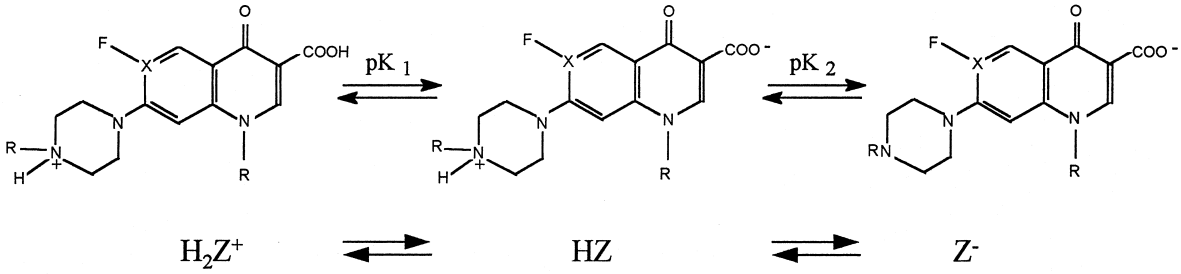

Fig. 2. Protolytic equilibria of quinolones.

of 0.5103 and a_0B is 1.5, in accordance with IUPAC [22–24].

In order to study the influence of pH on the electrophoretic behaviour of substances, we can relate pH, pK_a and electrophoretic mobility. The relationships are based on the principle that a solute has its maximum electrophoretic mobility when it is fully ionized, has no mobility in its neutral form, and has an intermediate mobility in the pH region surrounding its pK_a [25]. So, when an acid, H_2Z^+ is deprotonated, the electrophoretic mobility, m_e, in a determinate concentration of the buffer is given by $m_e = x_a m_a$, where m_a and x_a are electrophoretic mobility and the fraction of the fully protonated species, H_2Z^+, respectively, obtaining the following equation:

$$m_e = \frac{[H_2Z^+]}{[HZ] + [H_2Z^+]} \cdot m_a \quad (4)$$

Eq. (4) can be rewritten as:

$$\frac{[HZ]}{[H_2Z^+]} = \frac{m_a - m_e}{m_e} \quad (5)$$

Substituting Eq. (5) in Eq. (3) gives:

$$pK_1 = pH + \log \frac{m_e}{m_a - m_e} - \frac{A\sqrt{I}}{1 + a_0 B\sqrt{I}} \quad (6)$$

This equation allows prediction of the influence of the pH on the electrophoretic behaviour of quinolones and also permits the determination of the first dissociation constant of the quinolones by measuring the electrophoretic mobility at different pH. This model requires the experimental determination of m_a and it is called the direct model [9,10].

Analogous expressions can be deduced in basic media [9,25] that permit the determination of the second dissociation constants of the quinolones (Fig. 2). Thus, considering that the electrophoretic mobility is maximum for species Z^-, (Fig. 2), in the pH region surrounding the pK_2, $m_e = x_b m_b$, where m_b and x_b are the electrophoretic mobility and the fraction of the fully deprotonated species, Z^-, we obtain the expression:

$$pK_2 = pH - \log \frac{m_e}{m_b - m_e} + \frac{A\sqrt{I}}{1 + a_0 B\sqrt{I}} \quad (7)$$

In order to apply this expression, m_b is determined experimentally for each substance.

Flumequine only has one dissociation constant due to the deprotonation of the carboxylic group, Fig. 1. The dissociation equilibrium ($HZ \leftrightarrow Z^- + H^+$) is the same as that considered in the second dissociation constant of the other quinolones studied, so Eq. (7) can also be used to relate the pK_a value of Flumequine, pH and electrophoretic mobilities.

Most quinolones have two close pK_a values [1,19], so they could not be considered independently. Here we develop a model that considers pK_1 and pK_2 values simultaneously to investigate the influence of pH on the electrophoretic mobility of substances and permits the determination of the pK_1 and pK_2 values.

The model considers that, in general, the electrophoretic mobility of the substance can be written according to the mobility and fraction of its species.

$$m_e = x_{H_2Z^+} m_{H_2Z^+} + x_{HZ} m_{HZ} + x_{Z^-} m_{Z^-} \quad (8)$$

where the term corresponding to the intermediate species is considered nil because the species HZ has no charge and migrates with the EOF.

Substituting terms $x_{H_2Z^+}$ and x_{Z^-} by their expressions for ampholytes and considering that $m_{H_2Z^+} = m_a$ and $m_{Z^-} = m_b$, we can write:

$$m_e = \frac{a_{H^+}^2 y_{HZ} y_{Z^-} m_a - K_1 K_2 y_{H_2Z^+} y_{HZ} m_b}{a_{H^+}^2 y_{HZ} y_{Z^-} + K_1 a_{H^+} y_{H_2Z^+} y_{Z^-} + K_1 K_2 y_{H_2Z^+} y_{HZ}} \quad (9)$$

In accordance with previous works reported about determination of activity coefficients of zwitterionic species, if we consider the activity coefficient of the species HZ as 1 [20,21] and taking into account that $y_{H_2Z^+} = y_{Z^-}$, Eq. (9) can be written as:

$$m_e = \frac{a_{H^+}^2 m_a - K_1 K_2 m_b}{a_{H^+}^2 + K_1 a_{H^+} y + K_1 K_2} \quad (10)$$

where m_a has the opposite sign to m_b. The determination of the pK_a values of the substances requires experimental values of m_e at different pH values, and initial values of m_a, m_b, K_1 and K_2 which are refined simultaneously in order to obtain the dissociation constants and mobilities of ionized

species. A similar equation is reported by Ishihama [12], although different approaches were used to consider the activity coefficients. Applying this simultaneous model, data pairs of m_e and pH in both acid and basic range are imported into the NLREG program [26].

3. Results and discussion

Norfloxacin, Danofloxacin, and Difloxacin have two relevant ionizable functional groups, which means that their acid–base chemistry involves two equilibria, the dissociation of the carboxylic group and the deprotonation of the N_4 of the piperazine ring, placed at position 7 [19,27], (Fig. 2). In contrast, Flumequine has only one relevant ionizable functional group within the pH ranges of pharmaceutical or physiological importance [19,27]. In order to calculate the dissociation constants of quinolones by CE, values of electrophoretic mobilities, m_e, at different pH were obtained, injecting standard solution of each quinolone and acetone and using Eq. (1), as an average of at least three replicates.

In the determination of the first dissociation constants, buffers cover the pH range between 5.25 and 7.00 in a concentration of 0.050 *M*. The electrophoretic mobility values determined are shown in Fig. 3, which also shows the electrophoretic mobility of the fully protonated species for each quinolone, m_a, which was obtained by injecting the working solution of the quinolone and acetone at pH 2.00.

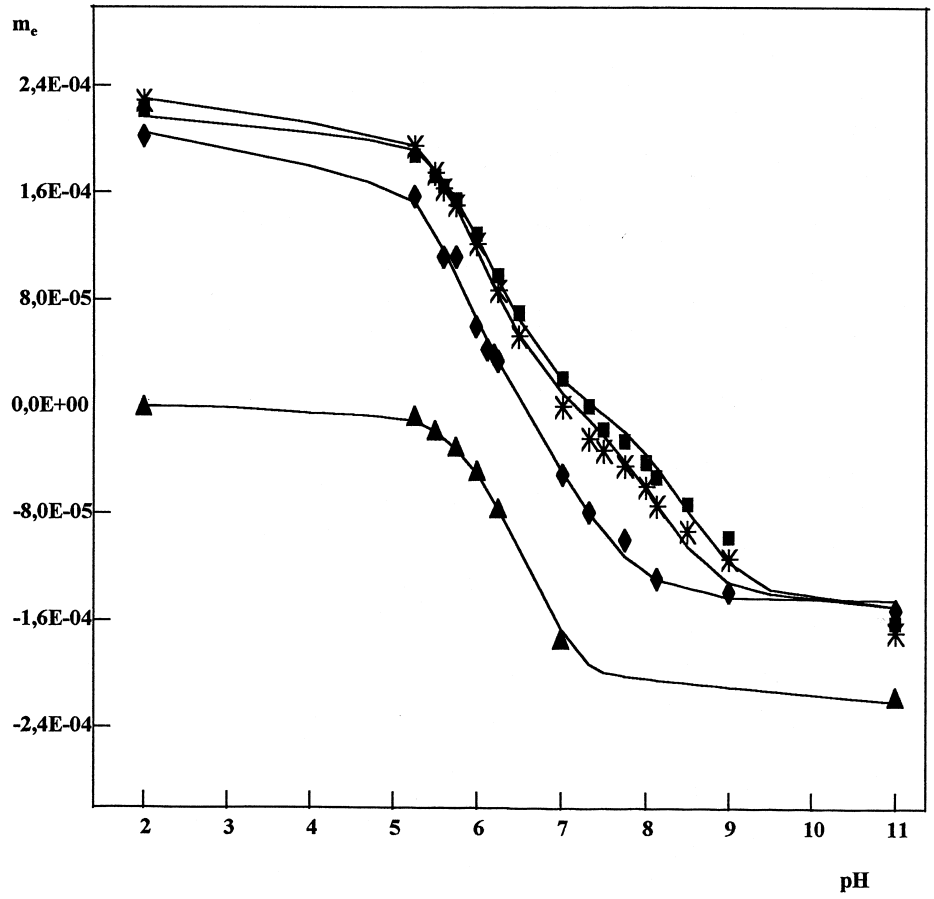

Fig. 3. Plot of electrophoretic mobility, m_e, vs. pH for Norfloxacin (*), Danofloxacin (■), Difloxacin (♦) and Flumequine (▲) with superimposed curve fits for the simultaneous model.

Values of electrophoretic mobility for Flumequine are also given in Fig. 3 with the mobility of its fully deprotonated species, which was obtained at basic pH. The values of m_e for three quinolones are positive because the quinolones have a net positive charge and are detected before the EOF marker. On the other hand, Flumequine was detected after acetone, so the values of m_e are negative.

In Fig. 3 electrophoretic mobilities of the quinolones at basic pH are also presented. The range of pH buffers, considered in the determination of pK_2, are between 7.02 and 9.00. Moderately high ionic strength buffers are desirable for the suppression of ionic interactions between charged analyte ions and ionized silanol groups on the capillary wall. However, high concentrations of buffers may overcome the capillary thermostatting capability of the system [28], so low concentration, 0.025 *M*, was chosen for working in basic media. For the determination of pK_2 values, electrophoretic mobility values of fully deprotonated species were obtained with a phosphate buffer at pH 11.0 and with borate buffer at pH 10.5. The concordance of the electrophoretic mobility values obtained with both buffers assures that deprotonated quinolone species is quantitatively formed. Also, this concordance indicates an effectively dissipation of generate Joule heating using phosphate buffer for which intensity is three times higher than intensity obtained for borate buffer. In order to verify the thermostatting capacity of the system we have proved the linearity of the plot of current vs. voltage (from 0 to 30 kV), at different pH. In all cases the Ohm's law is fulfilled and regression coefficients are greater than 0.997.

Table 1 shows, as an example, the pK_1 value obtained using the direct model for Norfloxacin according to Eq. (6). This table shows the dissociation constants for phosphoric acid, the values of the parameters of Debye–Hückel, a_0B and A [22–24], the value of mobility of fully protonated species m_a (obtained experimentally). This table also shows, for each pH, the value of ionic strength, I, the logarithm of activity coefficient of protonated species, log $y_{H_2Z^+}$, the electrophoretic mobility, m_e, and the pK_1 values obtained. Using this model, for each data pair pH $- m_e$ a pK value is obtained, so the mean value has been considered as a definitive result (Table 1).

Using the simultaneous determination model, data pairs pH–m_e over the whole range of pH are used. Initial values for electrophoretic mobility of fully protonated and deprotonated species, m_a, m_b and pK_1 and pK_2 are necessary in order to apply this model. The definitive values for these parameters are obtained from the fit of this model.

Values of pK_1 and pK_2 determined for all quinolones from the application of the two models are collected in Table 2. For each value of pK_a the respective standard deviation is also given. As can be seen in Table 2, there are no significant differences between the pK_1 values determined by the two models except for Difloxacin. So, the first dissociation constant of quinolones has been calculated as an

Table 1
pK_1 determination of Norfloxacin by means of the direct model

pK_1 (H_3PO_4)	pK_2 (H_3PO_4)	pK_3 (H_3PO_4)	A	a_0B	m_a
2.15	7.21	12.33	0.5103	1.500	$2.29\cdot10^{-4}$
pH	I	log $y_{H_2Z^+}$		m_e	pK_1
5.26	$5.11\cdot10^{-2}$	$-8.61\cdot10^{-2}$		$1.95\cdot10^{-4}$	5.93
5.50	$5.19\cdot10^{-2}$	$-8.66\cdot10^{-2}$		$1.75\cdot10^{-4}$	5.92
5.60	$5.24\cdot10^{-2}$	$-8.69\cdot10^{-2}$		$1.63\cdot10^{-4}$	5.90
5.75	$5.33\cdot10^{-2}$	$-8.75\cdot10^{-2}$		$1.51\cdot10^{-4}$	5.95
6.00	$5.58\cdot10^{-2}$	$-8.90\cdot10^{-2}$		$1.22\cdot10^{-4}$	5.97
6.25	$5.99\cdot10^{-2}$	$-9.13\cdot10^{-2}$		$8.66\cdot10^{-5}$	5.94
6.50	$6.63\cdot10^{-2}$	$-9.48\cdot10^{-2}$		$5.32\cdot10^{-5}$	5.89
				pK_1	5.93
				σ_{n-1}	0.03
				n	7

Table 2
pK_1, pK_2 and m_a, m_b ($\cdot 10^4$ $cm^2\ V^{-1}\ s^{-1}$) values of quinolones obtained from the different models[a]

Quinolone	Direct model		Simultaneous model		Definitive pK_a values
	pK_1	m_a (exp)	pK_1	m_a	pK_1
Norfloxacin	5.93 (0.03)	2.29 (0.1%)	5.93 (0.05)	2.29 (0.08)	5.93 (0.05)
Danofloxacin	6.01 (0.06)	2.22 (0.3%)	6.06 (0.05)	2.17 (0.06)	6.07 (0.06)
Difloxacin	5.51 (0.06)	2.02 (0.1%)	5.66 (0.04)	2.05 (0.06)	5.66 (0.04)
	pK_2	m_b (exp)	pK_2	m_b	pK_2
Norfloxacin	8.33 (0.11)	−1.68 (0.8%)	8.22 (0.07)	−1.50 (0.08)	8.22 (0.07)
Danofloxacin	8.56 (0.06)	−1.59 (0.4%)	8.56 (0.07)	−1.51 (0.07)	8.56 (0.07)
Difloxacin	7.44 (0.05)	−1.53 (0.6%)	7.24 (0.06)	−1.45 (0.04)	7.24 (0.06)
	pK_a	m_b (exp)	pK_a	m_b	pK_a
Flumequine	6.65 (0.09)	−2.12 (0.5%)	6.61 (0.03)	−2.21 (0.04)	6.63 (0.09)

[a] Standard deviations values are quoted in parentheses.

average of the pK_1 values obtained for each model, while for Difloxacin, because of their close pK_1 and pK_2 values the result obtained from the simultaneous determination have been chosen [12].

For the determination of second ionization constants, the models are applied in a similar way as for the pK_1 determination, using data pairs pH–m_e in basic media. The values of pK_2 obtained for Norfloxacin and Difloxacin using the simultaneous model are slightly lower than those obtained considering two independent equilibria. Considering this fact, pK_2 from the simultaneous model has been chosen, since this model seems to be the most suitable because it uses a high number of experimental data. It also allows the determination of pK_1 and pK_2 at a time, considering the dependence between the two dissociation constants.

In the case of Flumequine, with only one protolyte equilibrium, the pK_a values obtained from the two models are similar, as expected and we chose the mean value.

Table 2 also shows mobilities of the fully protonated species, m_b, obtained experimentally in order to compare these values with the obtained ones by the fitting of the data pairs $m_e - pH$ by simultaneous model. There is no significant difference between calculated and experimental values.

The first dissociation constant associated with the carboxylic acid function of the quinolones was higher than those generally observed for carboxylic acids ($pK \approx 5$). This decrease in acidity can be attributed to an intramolecular H-bond formation with the neighbouring keto function resulting in stabilization of the protonated species [29]. The formation of an intramolecular hydrogen bond is supported by UV and IR data [30].

There are a few pK values of quinolones reported in the literature [1,5,6,19,31], most of which have been determined in mixtures of solvents. In order to test the validity of the electrophoretic method for the determination of dissociation constants, the experimental data of pK_a has been compared with the literature pK_a values available of quinolones, determined by potentiometry in acetonitrile–water mixtures with low concentration of acetonitrile. These values are for Norfloxacin $pK_1 = 6.22$ in acetonitrile–water with 5.5% (w/w) of acetonitrile and $pK_2 = 8.48$ with 10% acetonitrile and also in this medium $pK_a = 6.90$ for Flumequine. As can be seen in Table 2, the electrophoretic pK_a values in water are lower than the corresponding pK_a values obtained by potenciometry in 5.5 and 10% (w/w) acetonitrile in water mixtures, just as can be expected, since in general, pK_a values lightly increase with the percentage of acetonitrile [6]. This fact and the low standard deviations obtained in applying the different models prove that CE is very suitable method for the determination of the dissociation constants.

Fig. 3 shows the experimental m_e values vs. pH in the range of pH between 2.00 and 11.0 for the quinolones studied. In this figure theoretical curves obtained by application of the simultaneous model are superimposed on the experimental data, showing a very good fit between Eq. (9) and the data of mobility.

The curve for Flumequine shows an inflection point that correspond to the only protolyte equilibrium of the substance, while Danofloxacin and Norfloxacin show two clear inflection points because of their two equilibria. However, although Difloxacin has two acid–base functional groups, it only shows one inflection point because of their close pK_1 and pK_2 values. For this reason the most suitable model for the determination of the dissociation constant as well as for prediction of the influence of pH on electrophoretic behaviour of substances is the simultaneous model.

Fig. 3 shows that for quinolones with two protolyte equilibrium, electrophoretic mobility varies from a positive to negative values. This is a common behaviour for ampholyte compounds which have two protolyte equilibrium. When pH values are lower than pK_1, quinolones have a positive net charge and migrate faster than the electroosmotic flow marker, which gives positive mobility values. When pH values are comprised between pK_1 and pK_2, the major form is the zwitterionic one, so quinolones migrate with electroosmotic flow and electrophoretic mobilities values are nil. At pH values greater than the pK_2, quinolones have a negative net charge and are detected after the electroosmotic flow marker, so mobility values are negative at these pH values. In the case for Flumequine, when pH values are lower than the pK_a, Flumequine is uncharged, so it comigrates with acetone and has a nil electrophoretic mobility value. At pH values higher than pK_a, Flumequine shows a negative charge due to the deprotonation of the carboxylic group, so this quinolone is detected after acetone and has a negative mobility value.

As can be seen in Fig. 3, the simultaneous model represented by Eq. (9) has two advantages. First, it allows the determination of pK_a of substances from m_e–pH data pairs and on the other hand permits the prediction of the effect of pH on electrophoretic behaviour of analytes. Thus, once pK_a is known, prediction of the optimum pH for the separation in electrophoretic methods is possible, using a minimum number of experimental measurements.

Acknowledgements

Financial support of DGICYT (Project PB95-0966) of the Spanish Government is gratefully acknowledged.

References

[1] D.C. Hooper, J.S. Wolfson, Quinolone Antimicrobial Agents, 2nd ed, American Society for Microbiology, Washington, DC, 1993.
[2] N. von Rosenstiel, D. Adam, Drugs 47 (1994) 872.
[3] D.A. Volmer, B. Mansoori, S.J. Locke, Anal. Chem. 69 (1997) 4143.
[4] Diario Oficial de las Comunidades Europeas (DOCE), 2377/90 L224, 991, 2608, 18 August 1990.
[5] K. Takacs-Novak, B. Noszal, I. Hermecz, G. Kereszturi, B. Podanyi, G. Szasz, J. Pharm. Sci. 79 (1990) 1023.
[6] J. Barbosa, I. Marqués, G. Fonrodona, D. Barrón, V. Sanz-Nebot, Trend. Anal. Chem. 16 (1997) 140.
[7] J.A. Hernández-Arteseros, J. Barbosa, R. Compañó, M.D. Prat, Chromatographia 48 (1998) 251.
[8] R. Huhn, S. Hoffsletter-Kuhn, Capillary Electrophoresis: Principles and Practice, Springer, 1993.
[9] J.A. Cleveland Jr., M.H. Benko, S.J. Gluck, Y.M. Walbroehl, J. Chromatogr. A 652 (1993) 301.
[10] J. Cai, J.T. Smith, Z.E. Rassi, J. High Resolut. Chromatogr. 15 (1992) 30.
[11] S.J. Gluck, K.P. Steele, M.H. Benko, J. Chromatogr. A 745 (1996) 117.
[12] Y. Ishihama, Y. Oda, N. Asakawa, J. Pharm. Sci. 83 (1994) 1500.
[13] J. Barbosa, I. Toro, V. Sanz-Nebot, J. Chromatogr. A 823 (1998) 497.
[14] J. Barbosa, R. Bergés, V. Sanz-Nebot, J. Chromatogr. A 823 (1998) 411.
[15] J. Barbosa, R. Bergés, V. Sanz-Nebot, J. Chromatogr. A 719 (1996) 27.
[16] J.L. Beckers, F.M. Ackermans, J. Chromatogr. 537 (1991) 407.
[17] R.M. McCormick, Anal. Chem. 60 (1988) 2322.
[18] D.R. Baker, Capillary Electrophoresis, Wiley, New York, 1995.
[19] J. Barbosa, R. Bergés, I. Toro, V. Sanz-Nebot, Talanta 49 (1997) 1271.
[20] R. Rodriguez-Raposo, L. Fernández-Merida, M.A. Esteso, J. Chem. Thermodyn. 26 (1994) 1121.

[21] R.N. Roy, C.P. Moore, J.A. Caristen, W.S. Good, P. Harris, J.M. Rook, L.N. Roy, K.M. Kuhler, J. Solut. Chem. 26 (1997) 1209.
[22] T. Mussini, A.K. Covington, P. Longhi, Pure. Appl. Chem. 57 (1985) 865.
[23] M. Filomena Camoes, M.J. Guiomar Lito, M.I.A. Ferra, A.K. Covington, Pure. Appl. Chem. 69 (1997) 1325.
[24] J. Barbosa, V. Sanz-Nebot, Anal. Chim. Acta 244 (1991) 183.
[25] S.J. Gluck, J.A. Cleveland, J. Chromatogr. A 680 (1994) 49.
[26] P.H. Sherood, NLREG program, 1997.
[27] J. Barbosa, G. Fonrodona, I. Marqués, S. Butí, I. Toro, Trend. Anal. Chem. 16 (1997) 104.
[28] J.P. Landers, Handbook of Capillary Electrophoresis, CRC Press, Boca Raton, FL, 1994.
[29] J. Barbosa, D. Barrón, R. Bergés, V. Sanz-Nebot, I. Toro, J. Chem. Soc., Faraday Trans. 93 (1997) 1915.
[30] D.L. Ross, C.M. Riley, Int. J. Pharm. 83 (1992) 267.
[31] D.L. Ross, C.M. Riley, Int. J. Pharm. 63 (1990) 237.