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Prediction of electrophoretic mobilities in non-aqueous capillary electrophoresis
Optimal separation of quinolones in acetonitrile–water media

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Abstract

A model of electrophoretic behaviour is used to predict the optimum conditions for the separation of a series of quinolones zwitterionic substances in mixtures of acetonitrile–water up to 30% (w/w) of acetonitrile. The effect of pH, pK_a, the electrophoretic mobility of protonated and anionic species, and activity coefficients on the electrophoretic behaviour of quinolones is considered. The model proposed allows the resolution between substances in acetonitrile–water mixtures to be predicted from a few experimental data and thus permits one to obtain the best experimental conditions for separation methodologies.

Keywords: Electrophoretic mobility; Non-aqueous capillary electrophoresis; Buffer composition; Resolution prediction; Quinolones; Antibiotics

1. Introduction

Quinolones are synthetic antimicrobial agents widely used in both human and veterinary medicine. Their common skeleton is termed 4-oxo-1,4-dihydroquinoline, but they are better known under their generic name, 4-quinolones [1,2]. They were initially applied in the treatment of urinary tract infections, but now have broad-spectrum application because they are active against many Gram-positive and Gram-negative bacteria [1,3]. They are widely applied in the treatment and prevention of disease in food-producing animals and in commercially farmed fish. Although reports of rodent carcinogenicity have impeded the use of quinolones in aquaculture in the USA, they are used in Europe [4]. The wide application range and extensive use and misuse of quinolones in veterinary medicine represent a potential hazard because residues of these drugs may persist in edible tissues or milk. Proposed restrictive measures and the establishment of regulatory levels for quinolones have not yet been widely implemented in veterinary medicine, despite calls by regulatory agencies for restriction [5]. So the need to analyse quinolones in various biological tissues and fluids is obvious.

Most analytical methods for quinolones are based on liquid chromatography [6–9] but only a few methods have been developed for capillary electrophoresis (CE) [8,10–12], although this technique is firmly established as an efficient method for separating a wide variety of ionic species, as a consequence of their high selectivity and efficiency, and short analysis time [13–15].

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The use of non-aqueous solvents in general and binary water–organic solvent systems in particular extends the range of application of aqueous CE, rendering electrophoretic separation more versatile since it is possible to work in media with different dielectric constants, polarities, densities, viscosities and acid–base properties [16–18]. Other advantages of non-aqueous CE are improved solubility of analytes with low solubility in pure aqueous buffer solutions, and low operating currents when a voltage is applied [19]. As a result, less Joule heat is produced in non-aqueous CE conditions than in buffered aqueous systems, allowing much higher electric field strengths than are currently used in CE [15].

Various aqueous–organic mixtures have been used as separation media for CE [15,20]. Acetonitrile (MeCN) and its mixtures with water are widely used in non-aqueous CE, due to the excellent characteristics of the pure solvent. MeCN is a very weak base and a very weak acid and therefore it is a good differentiating solvent for both acids and bases [21]. Furthermore, it has low viscosity and good UV transparency, which make it ideal for non-aqueous CE [14,19]. The use of MeCN–water mixtures requires the correct measurement of pH in these media, which can be performed in a similar way to how they are performed in water using IUPAC standardisation rules, since in previous works standard pH values for the primary reference buffer solutions of the NIST scale were assigned in MeCN–water mixtures [22–24].

Here we test a model of electrophoretic behaviour of substances in MeCN–water mixtures, by establishing a relation between electrophoretic mobility and pH. The proposed model is used to predict the optimum pH for the separation of a series of eight quinolones in MeCN–water mixtures, from a few experimental data and with an estimation of the pK_a values of quinolones in the hydro-organic media studied.

2. Theory

The model of electrophoretic behaviour of the substances developed relates electrophoretic mobility with pH values measured in the MeCN–water mixtures used as electrophoretic media and takes into account the effect of activity coefficients and pK_a values. This relationship is based upon the principle that a compound shows its maximum mobility when it is completely ionised, has no mobility in its neutral form, and has intermediate mobilities at pH around its pK_a [25,26]. So, in general, we can calculate the electrophoretic mobility of a substance as a function of the mobility of each species and its molar fraction x_i. Most quinolones have two proton-binding sites [3,27,28] and we can consider a protonated species (H_2Z^+), a zwitterionic species (HZ) and a dissociated anionic species (Z^-). Then the electrophoretic mobility of the substance, m_e, is given by the following equation:

$$m_e = x_{H_2Z^+} m_{H_2Z^+} + x_{HZ} m_{HZ} + x_{Z^-} m_{Z^-} \quad (1)$$

where $m_{H_2Z^+}$, is the mobility of the fully protonated species and is represented as m_a, and $m_{Z^-}$is the mobility of the fully deprotonated species and is represented as m_b. Thus, considering the electrophoretic mobility of the zwitterionic species as zero [25], the dissociation constants of substances, the molar fraction of species and the activity coefficients [29,30], Eq. (1) can be written as:

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

where y is the activity coefficient of the species H_2Z^+ or Z^-.

Eq. (2) provides the electrophoretic mobility at any pH, if m_a, m_b, the dissociation constants of the substance and activity coefficient values are known. In this expression m_b has opposite sign to m_a and the activity coefficients can be calculated by Debye–Hückel equation taking into account that $y_{H_2Z^+} = y_{Z^-} = y$ [31,32].

For acidic substances that have only a dissociation equilibrium, such as flumequine, the same route is followed, to arrive at a similar equation:

$$m_e = -\frac{K_a m_b}{K_a + a_{H^+} y} \quad (3)$$

where m_b is negative and y values can be obtained through the Debye–Hückel equation [31,32].

The model, represented by Eqs. (2) and (3), allows one to calculate the electrophoretic mobility

of a substance at any pH, if the pK_a values are known. Furthermore m_a and m_b have to be determined and the activity coefficients calculated. pK_a values of substances are needed, besides a electrophoretic mobilities of protonated and deprotonated species, because of the effect of these parameters on electrophoretic behaviour.

3. Experimental

3.1. Apparatus

A Beckman P/ACE system 5500 (Beckman Instruments, Fullerton, CA, USA) was used in all the electrophoretic experiments. The system is equipped with a capillary cartridge, containing a 47 cm (40 cm from inlet to the detector)×75 μm I.D. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA). Detection was performed by a photodiode array detector monitoring each quinolone at 280 nm, except flumequine, which was monitored at 250 nm. When mixtures of quinolones were injected the detection was performed at 260 nm. The working voltage used was 20 kV. The temperature was held at 25°C using a fluorocarbon-based cooling fluid. Potentiometric measurements were performed with a Crison micropH 2002 meter (Crison Instruments, Barcelona, Spain) equipped with a ROSS electrode 8102 (Orion Research, Boston, MA, USA).

3.2. Capillary treatment

Capillary was first washed, prior to its initial use, with 1 *M* aqueous NaOH solution (30 min), followed by 30 min with water and 20 min of application of a voltage of 20 kV with the capillary filled with buffer solution. When the buffer was changed, the capillary was successively rinsed for 5 min with water, 20 min with 1 *M* aqueous NaOH, 20 min with water, 25 min with the new buffer and 20 min of voltage application. After each injection the capillary was washed with water (for 1 min) and with the working buffer (for 3 min). Furthermore, at the beginning of each day, we carried out a soft treatment with 0.1 *M* aqueous NaOH solution (15 min), water for 10 min, with the working buffer for 10 min, with a voltage of 20 kV applied for 8 min.

3.3. Chemical and reagents

Analytical-reagent grade chemicals were used, unless otherwise indicated. Phosphoric acid (85%), potassium hydrogenphthalate and acetone were supplied by Merck. Sodium hydroxide was obtained from Probus. Water, conductivity less than 0.05 μS cm^{-1}, was obtained using a Milli-Q water purification system (Millipore, Molsheim, France). Acetonitrile, HPLC-grade, was supplied by Baker. Norfloxacin and Flumequine were supplied by Sigma, sarafloxacin and difloxacin were supplied by Abbott, ciprofloxacin was obtained from Lasa Labs., enrofloxacin by Cenavisa, danofloxacin by Pfized and marbofloxacin by Vetoquinol Labs.

3.4. Preparation of solutions

The background solvent for the buffer solutions was prepared by mixing water and MeCN, in the appropriate amounts to obtain mixtures of 5.5%, 10% and 30% (w/w) of MeCN. In order to calibrate the pH meter before the measurement of pH in the different hydro-organic mixtures two buffers were used: sodium hydrogenphosphate plus potassium dihydrogenphosphate buffer (0.025 mol kg^{-1} of each component) was prepared in MeCN–water with 5.5%, 10% and 30% of MeCN. pH values of these reference buffer solutions are 7.15, 7.18 and 7.60 [22–24], respectively. Also, potassium hydrogenphthalate buffer (0.05 mol kg^{-1}) was prepared in each MeCN–water mixture. These standard reference solutions have pH values of 4.19, 4.32 and 5.02 [22–24] at 5.5%, 10% and 30% (w/w) of MeCN, respectively. Phosphate working solutions (25 m*M*) were obtained by diluting the concentrated solution of phosphoric acid with the appropriate MeCN–water mixture, and adjusting the pH by addition of NaOH. They were always prepared at 25 m*M* except while working at pH 11 for the basic mobility determination, in which case they were prepared at 18.7 m*M*, in order to avoid high intensities.

Working quinolone solutions were prepared in aqueous acetic acid 50 m*M* at a concentration of 50 μg ml^{-1}. The marker of electroosmotic flow used was acetone in 3% (v/v) added to every quinolone solution. The solutions used to obtain the mobilities of protonated and anionic species of each quinolone

only contained the quinolone and the neutral marker. The rest of solutions injected contained several combinations of quinolones, and the marker. Marbofloxacin was only studied in MeCN–water mixtures with 10% and 30% (w/w) of MeCN. All the solutions were filtered through a nylon membrane of pore diameter of 0.45 μm.

3.5. Procedures

The carrier electrolyte consisted of different MeCN–water mixtures with phosphate buffer. Proportions of MeCN were 5.5, 10 and 30% (w/w). For each pH assayed, working solutions of quinolones (50 μg ml^{-1}) were injected (3 s) in triplicate for several days, in order to obtain constant electrophoretic mobility values.

The pH of the mobile phase was measured, in accordance with IUPAC rules [31,33], taking into account the reference pH of NIST buffer solutions in MeCN–water mixtures, which were assigned in previous works [22–24]. Thus, the pH_x of a solution:

$$pH_x = pH_s + \frac{E_s - E_x}{g} \quad (4)$$

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

The activity coefficient can be calculated on the bases of an extrathermodynamic assumption, i.e., a form of the classical Debye–Hückel equation:

$$\log y_i = -\frac{z_i^2 A\sqrt{I}}{1 + a_0 B\sqrt{I}} \quad (5)$$

where A and B are the Debye–Hückel constants, a_0 is the ion size parameter in the solvent mixture, z_i is the valence of the ion and I is the ionic strength. In compliance with IUPAC rules [31,33], the values of A and a_0B are assigned at $T=298.15$ K by an extension of the Bates–Guggenheim convention [34] in terms of

$$A = 1.825 \cdot 10^6 (\epsilon_S T)^{-3/2} \rho_S^{1/2} \quad (6)$$

$$a_0 B = 1.5(\epsilon_w \rho_S / \epsilon_S \rho_w)^{1/2} \quad (7)$$

where ϵ is the dielectric constant, ρ is the density and the subscripts w and s refer to pure water and to the appropriate solvent mixture, respectively. Values of Debye–Hückel parameters, A and a_0B at 25°C, at different percentages of MeCN in water were reported in previous works [35,36]. The densities and dielectric constants of the appropriate solvent mixtures have been obtained from interpolation from known values in other percentages [35] and the ionic strength is obtained as usual [31,33].

Values of electrophoretic mobilities for each quinolone in each MeCN–water medium studied, were calculated using the equation [25]:

$$m_e = (m_{ap} - m_{EOF}) = \frac{L_C L_D}{V}\left(\frac{1}{t_{ap}} - \frac{1}{t_{EOF}}\right) \quad (8)$$

where L_C is the capillary total length, L_D is the length to the detector, V is the applied voltage (20 kV) and t_{ap} and t_{EOF} are the migration times of quinolone and acetone, respectively.

4. Results and discussion

In order to determine m_a and m_b, solutions containing only a single substance and the marker of electroosmotic flow were injected. To determine m_a, these solutions were injected at pH 2 in each MeCN–water mixture studied, that is to say 5.5%, 10% and 30% (w/w) of MeCN. Each quinolone was injected on several days until it showed a constant electrophoretic mobility (variation of less than $2\cdot10^{-10}$ m^2 V^{-1} s^{-1}).The same rule was followed for the determination of m_b, but working at pH 11 in each MeCN–water mixture. From each electropherogram, the apparent migration time of the quinolone, t_{ap}, and the migration time of the acetone, which is the electroosmotic flow marker, are obtained. Values of electrophoretic mobilities for each quinolone in each MeCN–water medium studied, were calculated using Eq. (8). m_a and m_b values obtained for the quinolones in each MeCN–water mixture studied are shown in Table 1. The values of m_a decreased when the percentage of MeCN increased, while the effect on m_b values was the opposite.

Knowledge of the dissociation constants is necessary to verify the model of electrophoretic behaviour of substances. The literature on pK_a values in hydro-

Table 1
Values of the electrophoretic mobilities ($\cdot 10^8$ m^2 V^{-1} s^{-1}) of the fully protonated (m_a) and deprotonated (m_b) species, in the MeCN–water mixtures, mean of a minimum of three replicates (relative standard deviations are indicated in parentheses)

Quinolone		% (w/w) MeCN		
		5.5	10	30
Danofloxacin	m_a	2.23 (0.2%)	2.07 (0.04%)	1.87 (0.3%)
	m_b	−1.49 (0.4%)	−1.49 (0.3%)	−1.35 (0.02%)
Norfloxacin	m_a	2.28 (0.2%)	2.13 (0.1%)	1.90 (0.3%)
	m_b	−1.58 (0.5%)	−1.57 (0.3%)	−1.43 (0.2%)
Ciprofloxacin	m_a	2.28 (0.1%)	2.11 (0.4%)	1.90 (0.08%)
	m_b	−1.55 (0.3%)	−1.57 (0.1%)	−1.42 (0.6%)
Sarafloxacin	m_a	2.12 (0.1%)	1.97 (0.1%)	1.77 (0.1%)
	m_b	−1.48 (0.8%)	−1.49 (0.3%)	−1.38 (0.2%)
Enrofloxacin	m_a	2.13 (1.3%)	2.00 (0.1%)	1.80 (0.2%)
	m_b	−1.46 (0.3%)	−1.44 (0.4%)	−1.38 (0.6%)
Difloxacin	m_a	2.09 (0.1%)	1.95 (0.1%)	1.76 (0.08%)
	m_b	−1.44 (0.3%)	−1.42 (0.3%)	−1.36 (0.5%)
Marbofloxacin	m_a	Not determined	2.10 (0.05%)	1.88 (0.1%)
	m_b	Not determined	−1.53 (0.1%)	−1.43 (0.3%)
Flumequine	m_b	−1.99 (0.3%)	−1.98 (0.2%)	−1.86 (0.1%)

organic mixtures is often scarce although for quinolones pK_a values in water [26,37–39] and in MeCN–water [3,27,40,41] have been previously determined. From these pK_a values in water and in different MeCN–water mixtures, an estimation of the pK_a values of quinolones in each MeCN–water medium studied can be made taking into account the variation of pK_a values with the percentage of MeCN [32,41]. The values used finally in this work are given in Table 2. The values in parentheses, indicative of standard deviation, were previously determined [3,26,41].

In this table can be observed that the pK_a values increase when increasing the amount of organic solvent in the mixture. This variation is stronger in pK_1 than in pK_2. This happens because dissociation of substances in MeCN–water is ruled by electrostatic interactions, as well as specific solute–solvent 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 MeCN content. For dissociation of the carboxylic acid of the quinolones, pK_1, the electrostatic interaction overwhelm the specific solvation and the pK_1 value increases with the percentage of MeCN [32,40]. Instead, in dissociation of a monocharged cation acid (such as the ammonium ions of the N_4 of piperazine ring of quinolones, pK_2) [3,27], there is no change in the number of charges ($HA^+ \leftrightarrow H^+ + A$) and the change in the dielectric constant of the medium does not affect the dissociation process. So, the pK_2 values of quinolones in MeCN–water mixtures show low changes than pK_1 in the range 0% (w/w) to 30% (w/w) of MeCN. Thus, the resulting effect in increasing the amount of MeCN in the media, is an approaching of the two pK_a of the quinolones, Table 2 [3,40,41].

Once the m_a and m_b values, the dissociation constants and the activity coefficients are known, the electrophoretic mobility of substances can be predicted by the proposed model for each substance at any pH. In our work we have obtained the theoretical

Table 2
Estimate values of pK_1 and pK_2 for quinolones studied

Quinolone		% (w/w) MeCN			
		0	5.5	10	30
Danofloxacin	pK_1	6.07 (0.06)[a]	6.3	6.5	7.5
	pK_2	8.56 (0.07)	8.6	8.6	8.9
Norfloxacin	pK_1	5.94 (0.05)	6.26 (0.05)	6.57 (0.06)	7.45 (0.05)
	pK_2	8.22 (0.07)	8.2	8.48 (0.03)	8.72 (0.02)
Ciprofloxacin	pK_1	5.86 (0.05)	6.13 (0.05)	6.3	7.0
	pK_2	8.24 (0.07)	8.2	8.38 (0.04)	8.41 (0.04)
Sarafloxacin	pK_1	5.62 (0.08)	5.8	6.0	6.7
	pK_2	8.18 (0.09)	8.2	8.2	8.5
Enrofloxacin	pK_1	5.88 (0.03)	6.1	6.3	7.0
	pK_2	7.74 (0.03)	7.8	7.8	8.0
Difloxacin	pK_1	5.66 (0.04)	5.9	6.1	6.8
	pK_2	7.24 (0.06)	7.2	7.3	7.5
Flumequine	pK_1	6.65 (0.09)	6.8	6.90 (0.04)	7.78 (0.02)

[a] Values with standard deviation associated, in parentheses, are obtained from the literature [3,26,41].

curves for all the quinolones studied. In this work has reported that changes in activity coefficient, y, are smaller than the changes produced by pK_a variation or m_a and m_b, even if the variation in y is ± 0.2, which corresponds to a working range of ionic strength of about $5 \cdot 10^{-1}$–$5 \cdot 10^{-3}$ M. The greatest variations in the mobility are found where the pH is close to pK_a values. In order to test the model, quinolone mixtures with acetone were injected in a wide range of pH, from pH 2 to pH 11, working at three percentages of MeCN [5.5%, 10% and 30% (w/w) of MeCN]. From the obtained electropherograms, the mobilities for each quinolone were calculated and compared with the model predictions.

As an example, Fig. 1 shows the comparison between experimental and predicted mobilities of sarafloxacin and difloxacin in the three MeCN–water mixtures studied, obtaining a good relationship between them. We have checked that similar results are obtained for the rest of quinolones in the three percentages of MeCN studied. Thus, experimental electrophoretic mobilities results for each quinolone agree well with the theoretical curves of variation of mobilities vs. pH.

The usefulness of these curves, on plotting all together, is that thus reveal the evolution of the electrophoretic behaviour of mixtures of quinolones with pH. Hence, at a determinate pH, we obtain the theoretical electropherogram, that is to say, the order of migration of the quinolones and the possibility of separating those substances when the mobilities are different enough. The obtained curves are plotted for all the quinolones studied in Fig. 2a for hydro-organic mixtures with 5.5% (w/w) of MeCN, in Fig. 2b for the 10% (w/w) of MeCN and in Fig. 2c for the 30% (w/w) of MeCN.

The theoretical curves in MeCN–water mixtures with 5.5% and 10% (w/w) of MeCN, Fig. 2a and b, show two inflexion points, in general due to the two dissociation constants of quinolones. For flumequine which has only one acid–base equilibrium and in the case of enrofloxacin and difloxacin, whose dissociation constants are very close (Table 2), only one inflexion point is observed. In MeCN–water with 30% (w/w) of MeCN, Fig. 2c, only sarafloxacin shows two inflexion points while for the rest of quinolones, with differences lower than 1.8 between pK_1 and pK_2 values (Table 2), the two inflexion

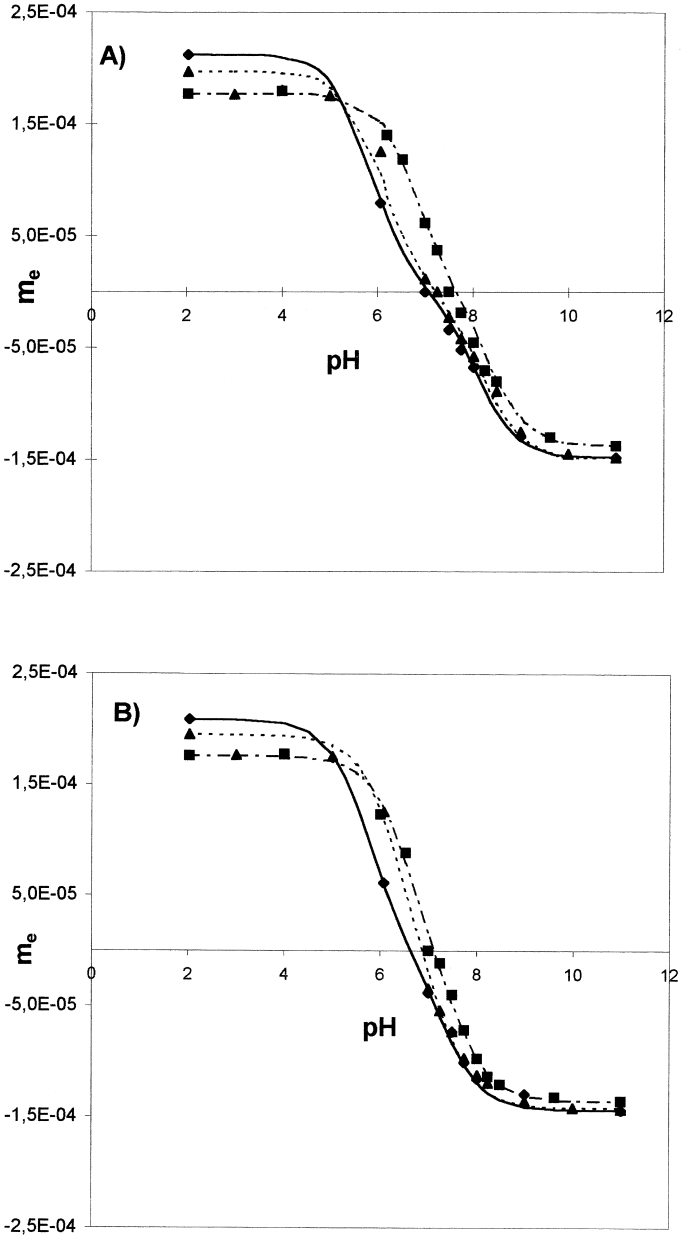

Fig. 1. Plot of experimental and predicted mobilities of sarafloxacin (A) and difloxacin (B) vs. pH at the studied percentages of MeCN. Symbols: Experimental m_e at 5.5% (w/w) of MeCN (♦). Predicted m_e at 5.5% (w/w) of MeCN (——). Experimental m_e at 10% (w/w) of MeCN (▲). Predicted m_e at 10% (w/w) of MeCN (- - -). Experimental m_e at 30% (w/w) of MeCN (■). Predicted m_e at 30% (w/w) of MeCN (— - —).

points merge into one. The curves shown in Fig. 2 are consistant with the experimental data of m_e obtained, as it is shown as example in Fig. 1 for sarafloxacin and difloxacin.

In order to predict the optimum pH range for the separation of quinolones, it is necessary to identify the pH values at which the differences between the mobilities of the studied substances are greatest and

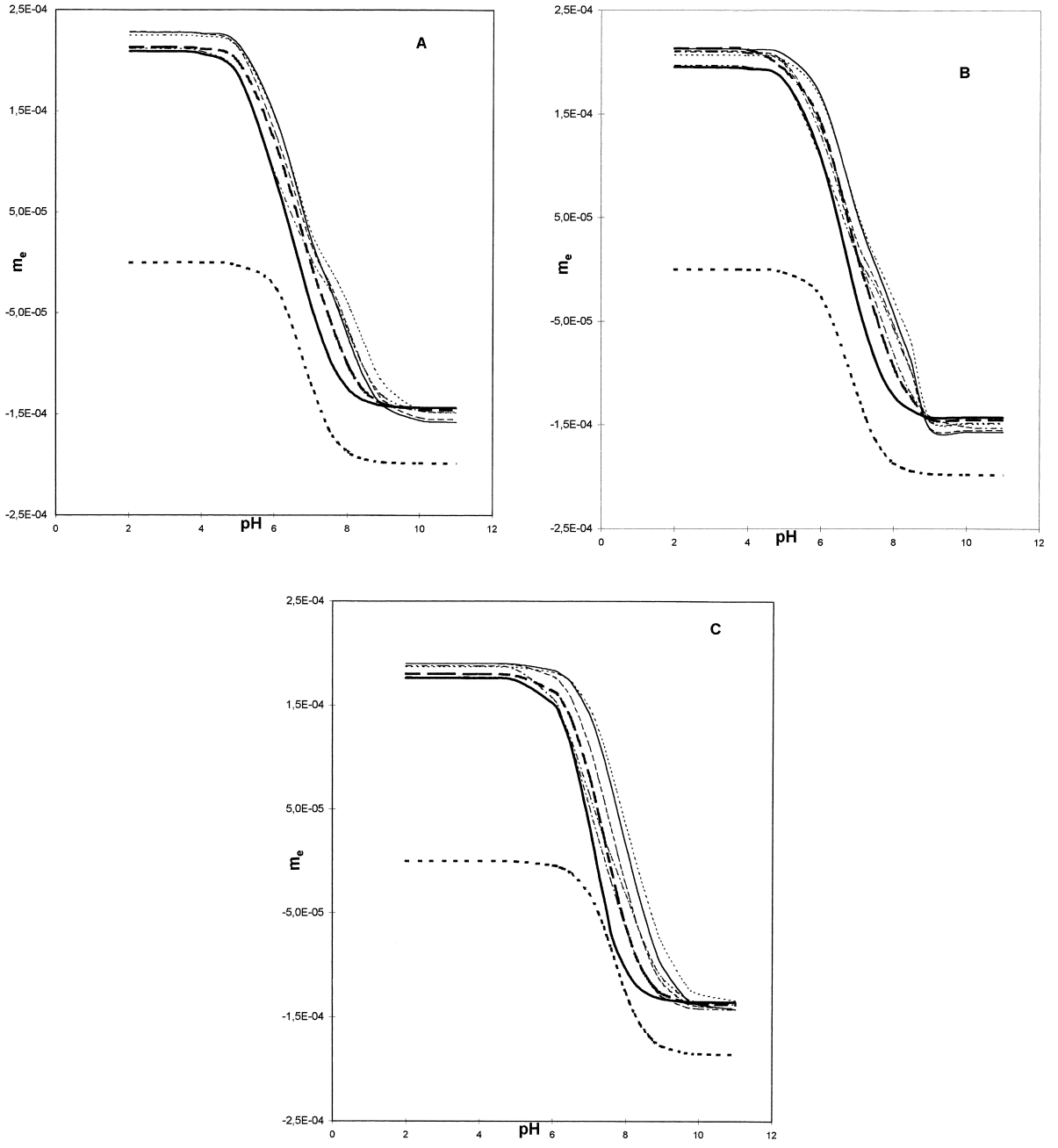

Fig. 2. Plot of electrophoretic mobility of quinolones vs. pH in MeCN–water mixtures with (A) 5.5% (w/w) of MeCN, (B) 10% (w/w) of MeCN, (C) 30% (w/w) of MeCN. Symbols: danofloxacin - - -; norfloxacin ——; flumequine - - -; ciprofloxacin — —; sarafloxacin — - - —; marbofloxacin — - —; enrofloxacin — — —; difloxacin ———.

hence, at which the substances migrate more separately. From Fig. 2a–c it is deduced that the best separation in all the percentages of MeCN studied is around pH 8, because at this pH the differences between the electrophoretic mobilities are greatest. Besides, these curves allow to predict the migration

order of the substances, because at a given pH, a quinolone with a higher electrophoretic mobility than other one will be detected earlier, that is to say at lower times.

On the other hand, the major or minor separation between two curves of two quinolones at the same pH, mean in the resulting electropherogram the major or minor distance between both peaks, and the separation from the $m_e = 0$ line means the distance from the electroosmotic flow marker.

However, the study of Fig. 2a–c suggests that although the best predicted separation is approximately between pH 7.75 and 8.5 for the three percentages of MeCN, there is no pH range where there is total separation for the eight quinolones. Quinolones that show the most similar mobilities are norfloxacin, ciprofloxacin and sarafloxacin expecting the co-migration of the three quinolones.

To verify these predictions, a mixture of the eight quinolones was injected at several pH values between 7.75 and 8.5. The results obtained after injections performed in 5.5% (w/w) of MeCN, agreed with the predictions of the model. The migration order was: acetone, danofloxacin, norfloxacin + ciprofloxacin + sarafloxacin migrated together, enrofloxacin, difloxacin and flumequine, as can be observed in Fig. 3 at pH 8.02. In MeCN–water mixtures with 10% (w/w) of MeCN, the migration order is that predicted in Fig. 2b, and the co-migration of norfloxacin, ciprofloxacin and sarafloxacin was obtained in accordance with the model. Marbofloxacin, with $pK_1 = 6.16$ and $pK_2 = 8.02$ in MeCN–water mixtures with 10% (w/w) of MeCN, leaves the capillary after difloxacin and before enrofloxacin. The best separation for the quinolones is obtained at pH 8, Fig. 4. The separation in MeCN–water with 10% (w/w) of MeCN is similar to that obtained with a percentage of 5.5% (w/w) of MeCN. The only difference is that in mixtures with 10% of MeCN, marbofloxacin is injected while in mixtures with 5.5% (w/w) of MeCN it is not.

The best separation obtained in MeCN–water medium with 30% (w/w) of MeCN at a pH of 8.50 is shown in Fig. 5 and agrees with the predictions of the curves shown in Fig. 2c. Working with this percentage of MeCN, separation is similar to the obtained ones at 5.5% and 10% but marbofloxacin and enrofloxacin migrate together.

However, in order to predict the optimum pH for the best separation, the parameter that should be studied is the resolution between pairs of adjacent peaks. The theoretical curves obtained previously can be used to calculate resolution between substances. Resolution can be expressed as [42]:

$$R_s = \frac{1}{4} \cdot N^{1/2} \cdot \frac{m_2 - m_1}{m_{avg} + m_{EOF}} \quad (9)$$

where m_i is the electrophoretic mobility of the solutes, m_{avg} is the average electrophoretic mobility of the solutes, m_{EOF} is the electroosmotic mobility and N is the efficiency. As can be observed in Eq. (9), it is essential to consider efficiency and electroosmotic mobility to calculate resolution. The separation efficiency used in Eq. (9) was the minimum value of $3 \cdot 10^4$ theoretical plates in the three MeCN–water media studied. Although better efficiencies would lead to greater resolution the ability to predict optimum pH would not be altered. Values of electrophoretic mobility are obtained from Eq. (3) for each substance in each percentage of MeCN and m_{EOF} can be evaluated from the study of m_{EOF} vs. pH. In the range of pH of interest (between 7 and 9) the variation of electroosmotic mobility with pH is virtually nil and values of $5 \cdot 10^{-8}$, $5.3 \cdot 10^{-8}$ and $4.1 \cdot 10^{-8}$ $m^2\ V^{-1}\ s^{-1}$, are used as average value of m_{EOF} in the respective media (5.5%, 10% and 30%). Predicted resolution has been calculated for some data pairs of substances for which separation are difficult. The studied pairs are sarafloxacin/ciprofloxacin, danofloxacin/ciprofloxacin and enrofloxacin/marbofloxacin.

Fig. 6 shows the predicted resolution for the pairs of substances studied. The variation of the resolution with pH for the pair sarafloxacin/ciprofloxacin is presented in Fig. 6a, showing that these substances present a better separation working with a 30% (w/w) of MeCN, while their separation is similar in the 5.5 and 10%. The resolution between danofloxacin and ciprofloxacin is plotted in Fig. 6b; the highest values with a 30% (w/w) of MeCN were obtained. Fig. 6c shows the predicted resolution between enrofloxacin and marbofloxacin showing the best resolution in working at a 10% (w/w) of MeCN. These predictions has been proved as can be observed in Figs. 3–5.

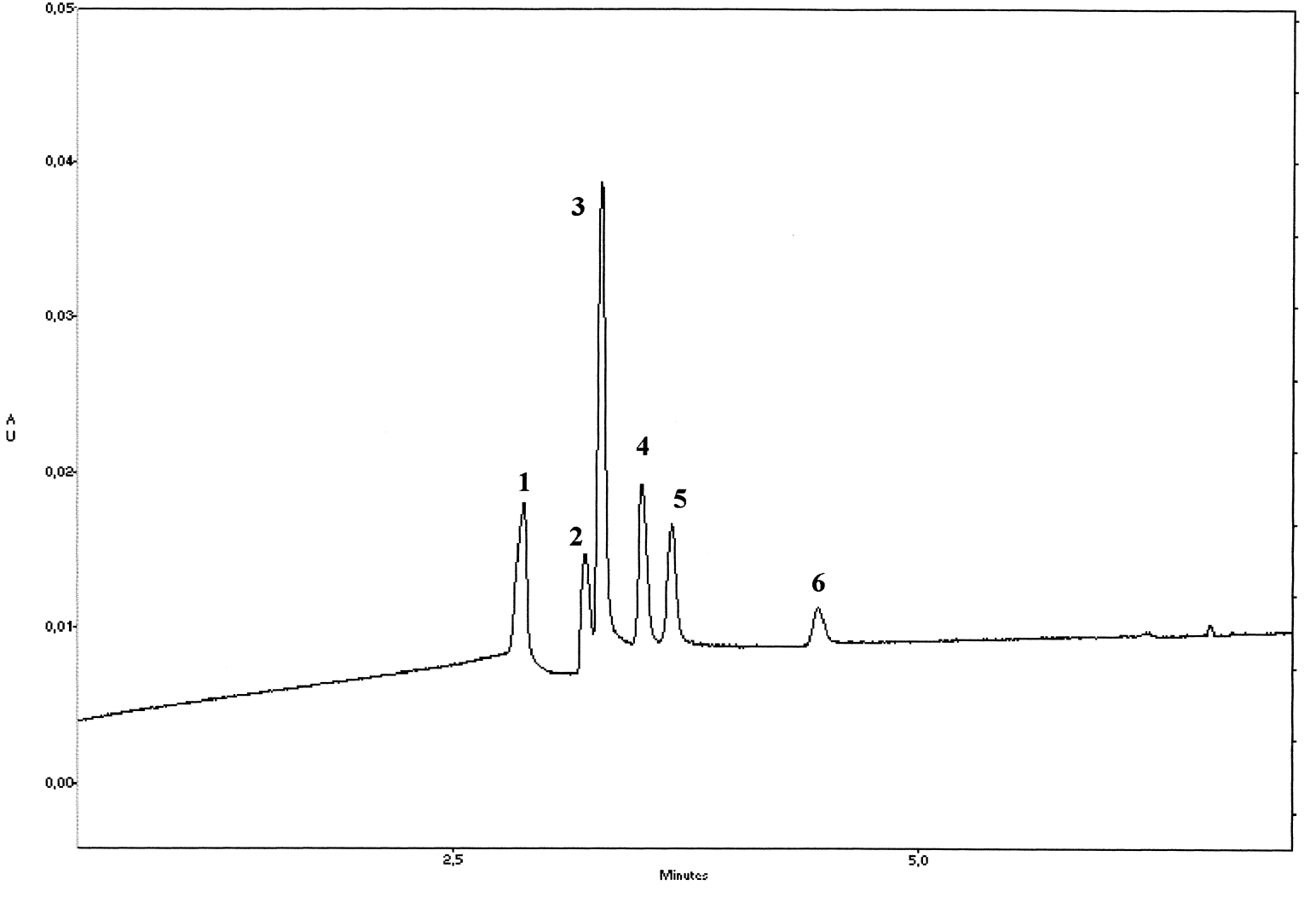

Fig. 3. Electropherogram of a mixture of quinolones using a fused-silica capillary tube 47 cm×75 μm I.D., 25 m*M* phosphate buffer, pH 8.02 in MeCN–water (5.5:94.5, w/w), 20 kV. Detection $\lambda = 260$ nm. Peaks: 1 = acetone, 2 = danofloxacin, 3 = norfloxacin + ciprofloxacin + sarafloxacin, 4 = enrofloxacin, 5 = difloxacin, 6 = flumequine.

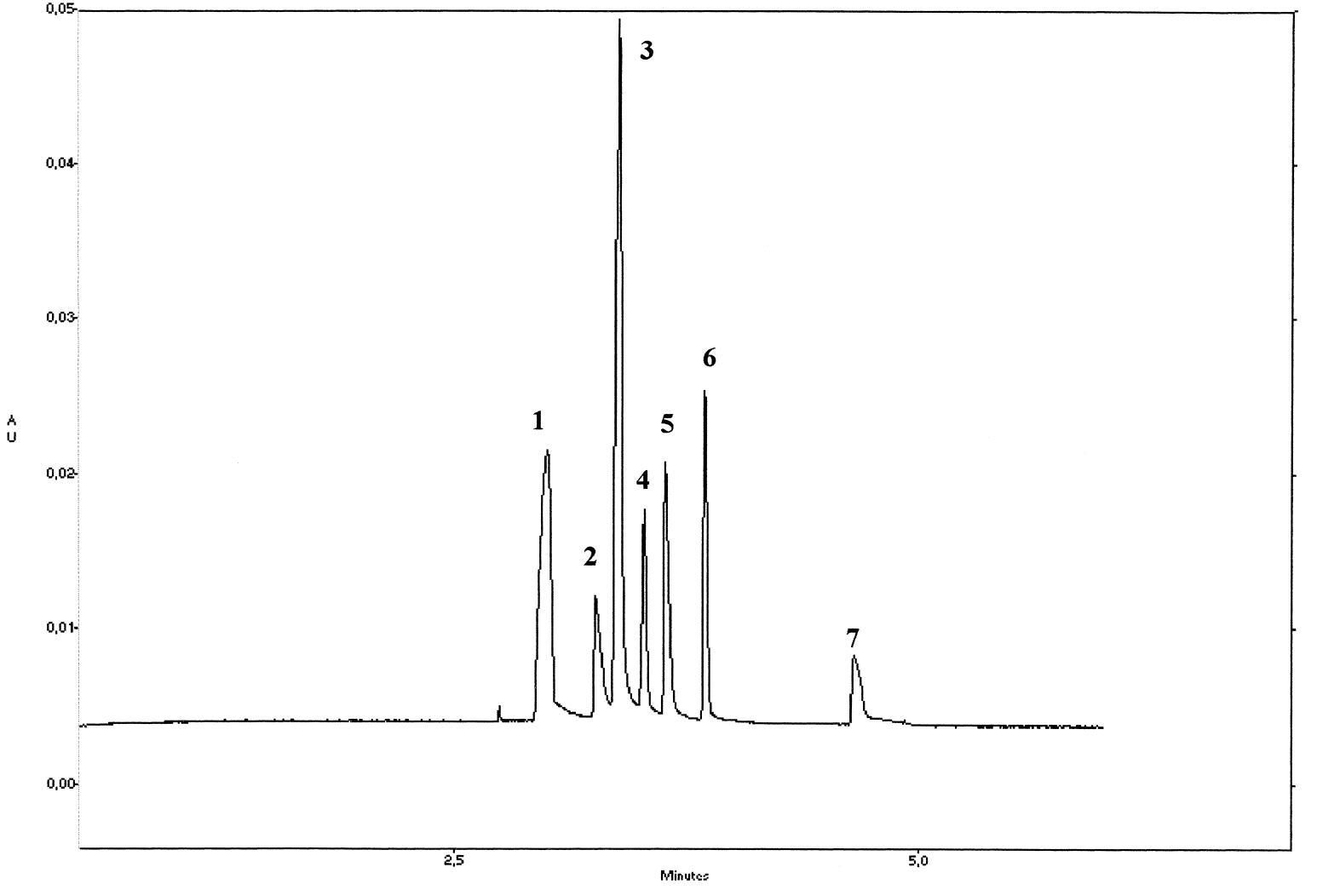

Fig. 4. Electropherogram of a mixture of quinolones in 25 m*M* phosphate buffer, pH 8.00 in MeCN–water (10:90, w/w). Peaks: 1 = acetone, 2 = danofloxacin, 3 = norfloxacin + ciprofloxacin + sarafloxacin, 4 = marbofloxacin, 5 = enrofloxacin, 6 = difloxacin, 7 = flumequine.

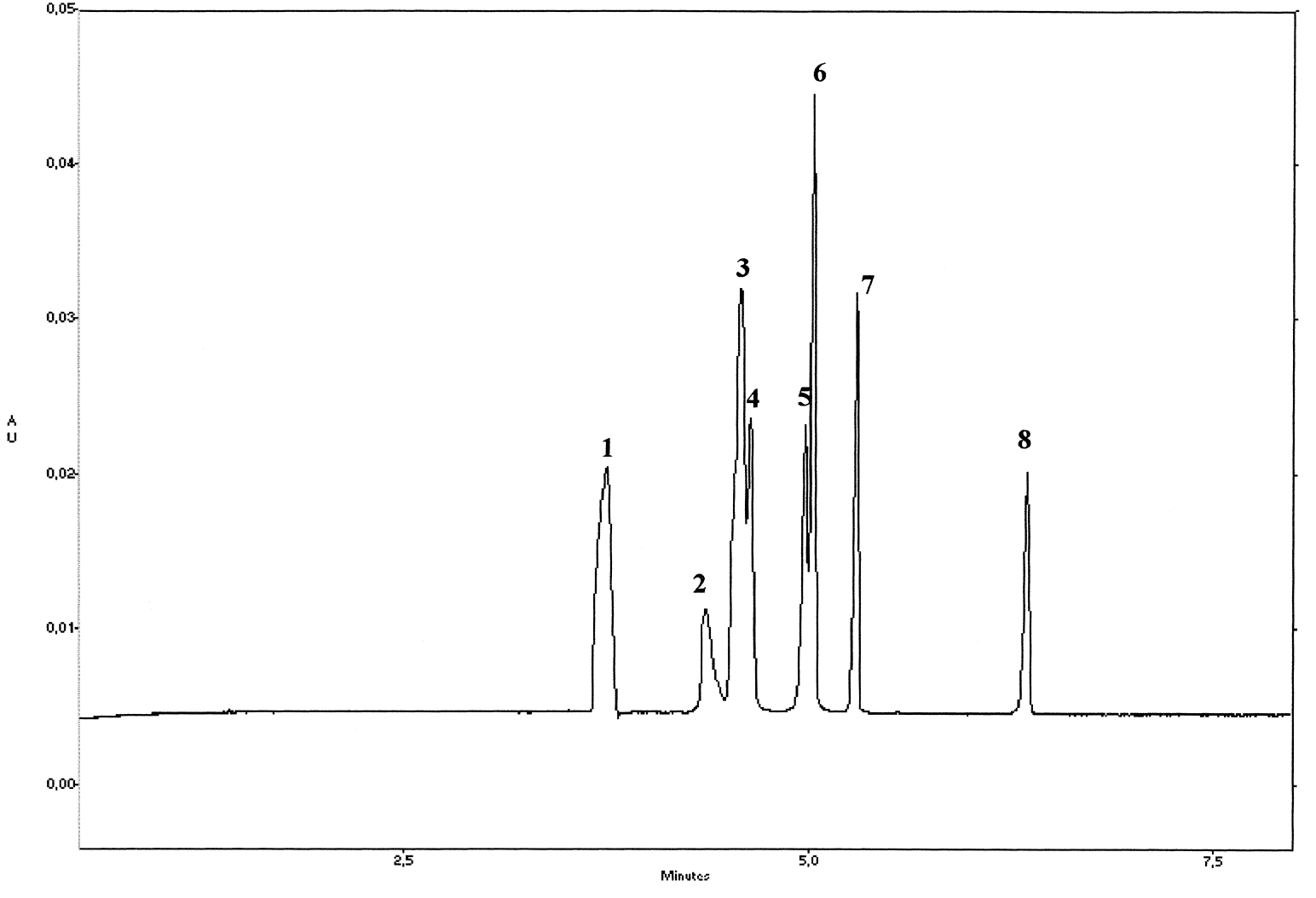

Fig. 5. Electropherogram of a mixture of quinolones in 25 m*M* phosphate buffer, pH 8.50 in MeCN–water (30:70, w/w). Peaks: 1=acetone, 2=danofloxacin, 3=norfloxacin+ciprofloxacin, 4=sarafloxacin, 5=marbofloxacin, 6=enrofloxacin, 7=difloxacin, 8=flumequine.

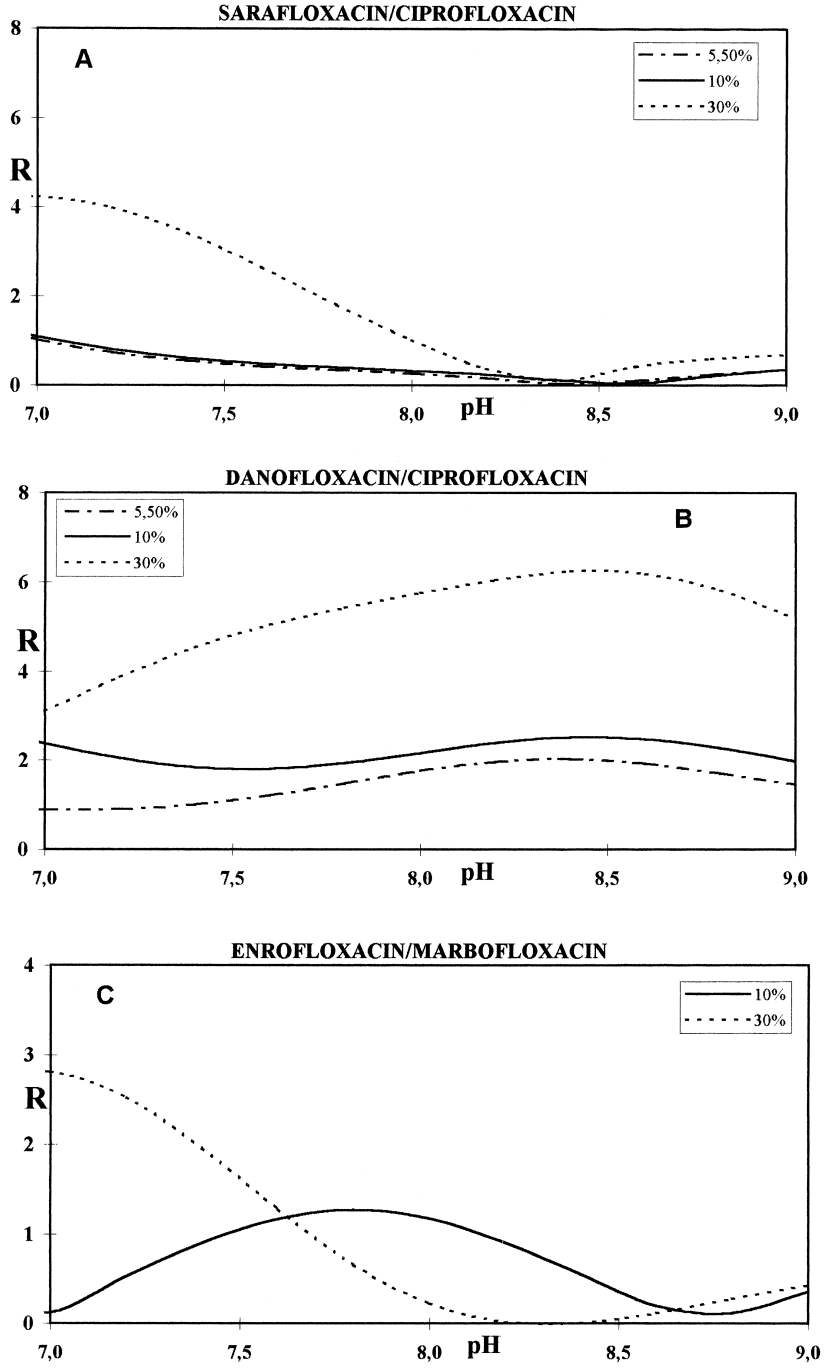

Fig. 6. Predicted resolution for some pairs of quinolones in MeCN–water media between pH 7 and pH 9. (A) Sarafloxacin/ciprofloxacin, (B) danofloxacin/ciprofloxacin, (C) enrofloxacin/marbofloxacin.

In conclusion, we have developed a tool that enables one to predict the best separation and allows the forecast of the electropherogram at any pH in hydro-organic media. The obtained results show that the proposed model could be a good, rapid, accurate and economic procedure to optimise the separation of a series of substances in CE using MeCN–water mixtures.

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