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Electrophoretic behavior and pK_a determination of quinolones with a piperazinyl substituent by capillary zone electrophoresis

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Abstract

Electrophoretic behavior and pK_a determination of six quinolones with a piperazinyl substituent, together with two quinolones without a piperazinyl substituent and 1-phenylpiperazine, were investigated by capillary zone electrophoresis. The results indicate that quinolones with a piperazinyl substituent involve three protonation/deprotonation equilibria. The results also suggest that the contribution of the zwitterionic species of these quinolones to the effective mobility may not be neglected. This is probably due to a slightly incomplete protonation of the piperazinyl moiety in the pH range of 6.0–8.0, compared with the complete dissociation of the carboxylic group. Consequently, the zwitterionic species of ciprofloxacin, in particular, is slightly negatively charged. With the aid of computer simulation, three pK_a values were determined for quinolones with a piperazinyl substituent, thus allowing us to rationalize precisely the influence of pH on the electrophoretic behavior of these compounds.

Keywords: pK_a determination; Quinolones; Antibiotics; Capillary zone electrophoresis

1. Introduction

Quinolones are used extensively as either antibacterial agents or antibiotics in both human and veterinary practices. As their antibacterial activity is pH-dependent and acid dissociation constants can be a key parameter for understanding chemical phenomena such as biological activity, biological transports, and drug delivery [1,2], a good knowledge of the protonation equilibria of quinolines is essential for a better understanding of their activity.

In recent years, the influence of pH on the electrophoretic behavior of quinolones in water and hydro–organic media has been examined by capillary electrophoresis (CE) [3–8], in which a single protonation equilibrium was considered for the piperazinyl substituent of those quinolones. As two protonation/deprotonation equilibria are involving in the piperazinyl substituent of these compounds [9,10], two pK_a values are expected to associate with the piperazinyl substituent. Therefore, it is thought that the protonation/deprotonation equilibria of those compounds are not accurately described in the literature. In view of the zwitterionic species of *p*-hydroxyphenylalanine being slightly negatively charged at pH 7.0 because of the incomplete compensation of the negative charge of the carboxylic group by the positive charge of the protonated amino group [11], it is of interest to find out whether the net charge of the zwitterionic species of quinolones with a piperazinyl moiety deviates from zero, because the dissociation of the carboxylic group may comparatively be stronger than the protonation of the piperazinyl moiety in the pH range 6.0–8.0.

Capillary zone electrophoresis (CZE) has proven to be a convenient and useful method for precise pK_a determination [2,12–23]. This method is based on the measurement of the electrophoretic mobility of the solute as a function of buffer pH and the analysis of the mobility curve using a mobility equation which describes appropriately the migration behavior of the solute in the pH range studied.

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In this study, the influences on the electrophoretic behavior of six quinolones with a piperazinyl substituent, together with two quinolones without a piperazinyl substituent and 1-phenylpiperazine, were investigated and their pK_a values were determined by CZE. The mobility equations which may properly describe the electrophoretic behavior of quinolones with a piperazinyl substituent were examined and accordingly, the pK_a values of six quinolones studied were determined. It is hoped that the protonation/deprotonation equilibria involving in these compounds can be better understood.

2. Experimental

2.1. Apparatus

All CE separations were performed on a Beckman P/ACE System MDQ equipped with a photodiode array detector for absorbance measurements at 214 nm (Beckman Coulter, Fullerton, CA, USA). Uncoated fused-silica capillary purchased from Polymicro Technologies (Phoenix, AZ, USA) was used. The dimensions of the capillary were 50.2 cm × 50 μm i.d. The effective length of the capillary was 40 cm. The CE system was interfaced with a microcomputer and a laser printer. System Gold software of Beckman was used for data acquisition. For pH measurements, a pH meter (Suntex Model SP-701, Taipei, Taiwan) with a precision of ±0.01 pH unit was used.

2.2. Chemicals and reagents

Ofloxacin, norfloxacin, enoxacin, and flumequine were obtained from Sigma (St. Louis, MO, USA). Nalidixic acid and 1-phenylpiperazine were purchased from Aldrich–Sigma (St. Louis, MO, USA). Lomefloxacin, ciprofloxacin, and pipemidic acid were obtained from local pharmaceutical suppliers. All other chemicals were of analytical reagent grade from various suppliers. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

Standard solutions of quinolones and 1-phenylpiperazine at a concentration of 10 μg/mL were prepared by dissolving analytes in an aqueous solution containing 10% (v/v) ethanol. The pH of a phosphate buffer was adjusted to the desired pH value by monitoring the pH of the solution with a pH meter while mixing various proportions of 50 mM trisodiumphosphate solution with the same concentration of phosphoric acid (50 mM). All buffer solutions, freshly prepared weekly and stored in a refrigerator before use, were filtered through a membrane filter (0.22 μm).

2.3. Electrophoretic procedure and operating conditions

When a new capillary was used, the capillary was washed 30 min with 1.0 M NaOH solution, followed by 30 min with deionized water at 25 °C. Before each injection, the capillary was prewashed for 3 min with running buffer. After each injection, the capillary was postwashed for 3 min with deionized water, 3 min with 0.1 M NaOH, and 5 min with deionized water to maintain proper reproducibility of run-to-run injections. Sample injections were done in a hydrodynamic mode over 5 s under a pressure of 1.0 psi at 25 °C. The measurements were run at least in triplicate to ensure reproducibility. The detection wavelength was set at 214 nm and a voltage of 20 kV was applied. Peak identification was conducted by spiking with the analyte to be identified. Ethanol was used as a neutral marker. The relative standard deviation of migration time is less than 0.6% (n = 5).

2.4. Mobility calculations

The electrophoretic mobility of analytes was calculated from the observed migration times with the equation:

$$\mu_{ep} = \mu - \mu_{eo} = \frac{L_d L_t}{V}\left(\frac{1}{t_m} - \frac{1}{t_{eo}}\right) \quad (1)$$

where μ_{ep} is the electrophoretic mobility of the analyte tested, μ is the apparent mobility of each quinolone, μ_{eo} is the electroosmotic mobility, t_m is the migration time measured directly from the electropherogram, t_{eo} is the migration time for an unchanged solute, L_t is the total length of capillary, L_d is the length of capillary between injection and detection, and V is the applied voltage.

3. Results and discussion

3.1. Influence of pH on electrophoretic behavior

Fig. 1 depicts the structures of eight quinolones studied (six with a piperazinyl moiety and two without the piperazinyl substituent). Fig. 2 shows the variation of the electrophoretic mobility of six quinolones with a piperazinyl moiety, together with flumequine, nalidixic acid, and 1-phenylpiperazine, as a function of buffer pH in the range 3.0–11.7. For clarity, the variations of the electrophoretic mobility of six quinolones with a piperazinyl substituent, i.e., ofloxacin and ciprofloxacin, enoxacin and norfloxacin, and lomefloxacin and pipemidic acid, are shown in Fig. 2A, B and C, respectively. Depending on the involvement of one, two, or three protonation/deprotonation equilibria, the mobility curves of quinolones without a piperazinyl group, 1-phenylpiperazine, and quinolones with a piperazinyl moiety exhibit different shapes. The sigmoidal shape of the mobility curves with negative values of the electrophoretic mobility were observed for flumequine and nalidixic acid, because these two compounds possess only one ionizable group. The shape of the mobility curve of 1-phenylpiperazine clearly indicates that two protonation/deprotonation equilibria are involved. Thus, in addition to a singly protonated species, there should exist a doubly protonated species. Quinolones with a piperazinyl substituent exhibit their substituent curves in a much complicated shape because these compounds possess

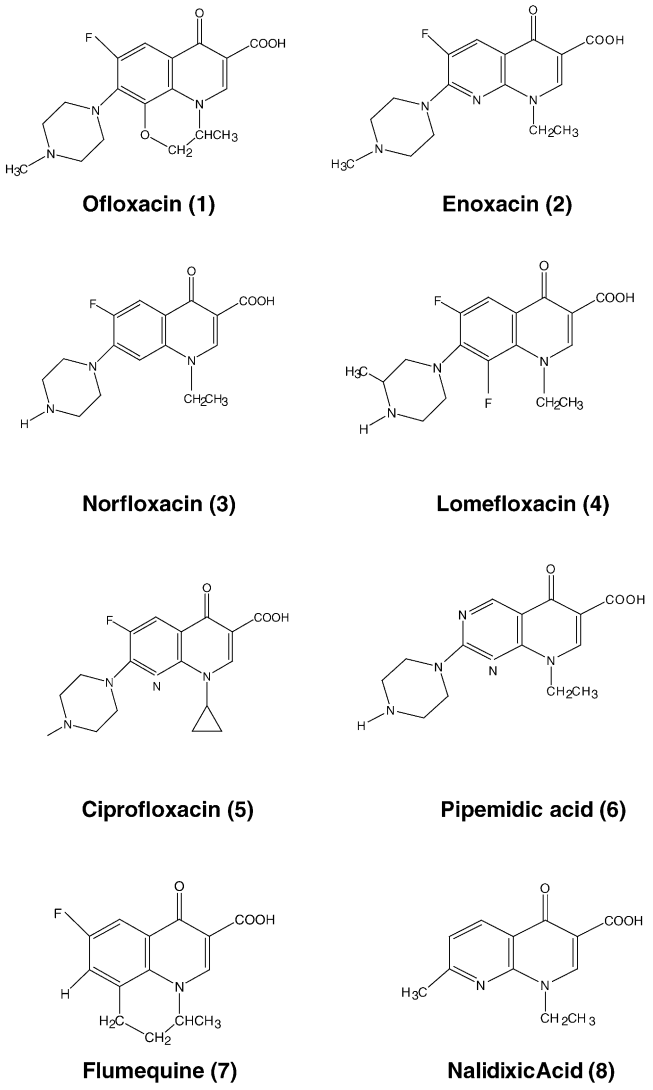

Fig. 1. Structures of the eight quinolones studied.

a piperazinyl substituent and a carboxylic group. Three protonation/deprotonation equilibria are expected and the electrophoretic mobilities of these compounds span from positive to negative values. As one of the inflection point is obscurely observed, these mobility curves can only be analyzed with the aid of computer simulation based on a mobility equation involving three protonation/deprotonation equilibria which will be discussed later in Section 3.2.

The fact that the negative values of the electrophoretic mobility of quinolones with a piperazinyl group are relatively less negative than those of the fully dissociated species of quinolones without a piperazinyl group, such as flumequine and nalidixic acid, in the pH region 5.0–10.0 may suggest that, in addition to the dissociated carboxylic group, there exists a protonated moiety in quinolones with a piperazinyl substituent. Thus, a zweitterionic species (HB^+A^-) exists and a deprotonation equilibrium occurs between the species HB^+A^- and BA^- for quinolones with a piperazinyl substituent in the pH region 7.0–10.0. On the other hand, in comparison with the nil values of the electrophoretic mobility of a fully protonated species of quinolones without a piperazinyl substituent, such as flumequine or nalidixic acid, at pH < 3.5, that the positive values of the electrophoretic mobility of quinolones with a piperazinyl group is relatively much larger than the negative values (actually the absolute values) of the electrophoretic mobility, at pH > 9.5, clearly indicate the existence of a doubly protonated species of quinolones with a piperazinyl substituent (H_2BAH^{2+}).

3.2. Determination of pK_a values

3.2.1. Mobility equation involving one protolytic equilibrium

As shown in Fig. 1, flumequine and nalidixic acid possess only one ionizable functional group. Thus, due to the disso-

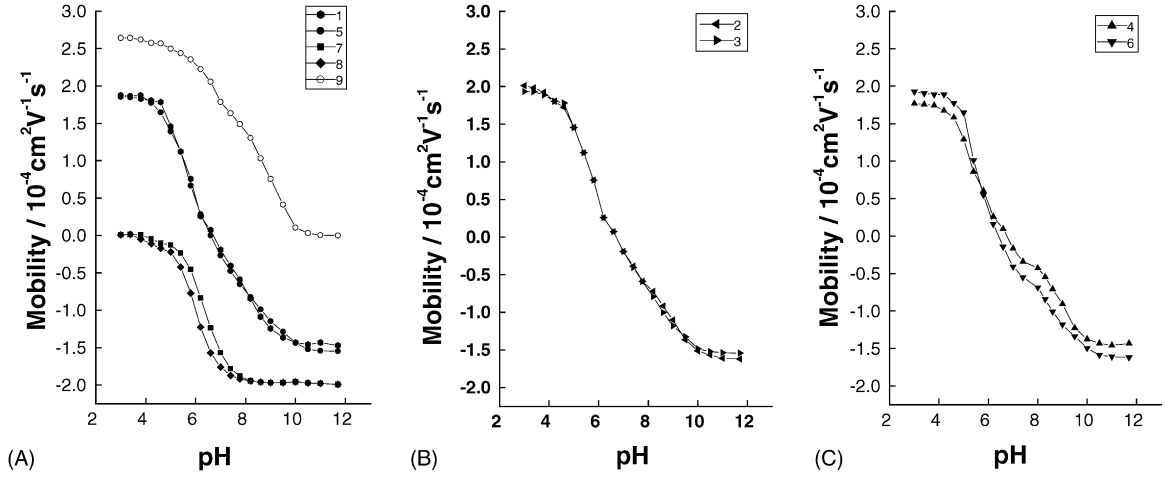

Fig. 2. Variations of the electrophoretic mobility of eight quinolones and 1-phenylpiperazine as a function of buffer pH in the range 3.0–11.7 using a phosphate buffer (50 mM). Capillary, 50.2 cm × 50 μm i.d; sample concentration, 10 μg/mL; detection wavelength, 214 nm. Curve identification: 1, ofloxacin (⬢); 2, enoxacin (◄); 3, norfloxacin (►); 4, lomefloxacin (▲); 5, ciprofloxacin (●); 6, pipemidic acid (▼); 7, flumequine (■); 8, nalidixic acid (⧫); 9, 1-phenylpiperazine (○).

Table 1
The pK_a values and limiting mobility data of quinolones and piperazine derivatives evaluated according to Eq. (4)

Analytes	Literature values[a]		pK_a values			Limiting mobility[b]			
	pK_a		pK_{a3}	pK_{a2}	pK_{a1}	μ_{BA}	$\mu_{H^+BA^-}$	μ_{HBAH^+}	$\mu_{H_2BAH^{2+}}$
Quinolones(with a piperazine substituent)									
Ofloxacin (1)	8.11	6.05	8.20 ± 0.02	6.20 ± 0.03	5.20 ± 0.06	−1.45 ± 0.01	−0.27 ± 0.01	0.95 ± 0.02	1.86 ± 0.01
Enoxacin (2)	8.50	6.00	8.80 ± 0.02	6.25 ± 0.05	5.05 ± 0.08	−1.60 ± 0.02	−0.43 ± 0.02	1.00 ± 0.03	2.00 ± 0.02
Norfloxacin (3)	8.38	6.22	8.45 ± 0.03	6.25 ± 0.04	5.00 ± 0.10	−1.55 ± 0.02	−0.38 ± 0.02	0.95 ± 0.03	1.95 ± 0.02
	8.22	5.94							
Lomefloxacin (4)	–	–	9.00 ± 0.03	6.25 ± 0.05	5.00 ± 0.10	−1.48 ± 0.01	−0.36 ± 0.02	0.86 ± 0.02	1.78 ± 0.01
Ciprofloxacin (5)	8.24	5.86	8.95 ± 0.04	6.35 ± 0.07	5.05 ± 0.15	−1.54 ± 0.02	−0.65 ± 0.03	0.95 ± 0.03	1.89 ± 0.02
	8.62	6.09							
Pipemidic acid (6)	8.18	5.42	8.90 ± 0.04	6.15 ± 0.06	5.25 ± 0.12	−1.61 ± 0.02	−0.60 ± 0.03	0.90 ± 0.03	1.95 ± 0.02
			pK_a			pK_a			μ_A-
Quinolones (without a piperazine)									
Flumequine (7)			6.61		6.50	6.35 ± 0.01			−1.97 ± 0.02
Nalidixic Acid (8)			5.95		6.01	6.00 ± 0.01			−1.97 ± 0.02
		pK_{a2}	pK_{a1}	pK_{a2}		pK_{a1}	μ_{BH^+}		$\mu_{BH_2^{2+}}$
Piperazine derivatives									
1-Phenylpiperazine		–	–	8.80 ± 0.02		6.30 ± 0.02	1.68 ± 0.02		2.58 ± 0.03
Piperazine		9.73	5.33	–		–	–		–

[a] Literature pK_a values of quinolones obtained from [3–5,7,8]; literature values of piperazine obtained from [9].
[b] Mobility in unit of /10^{-4} cm^2 V^{-1} s^{-1}.

ciation of the carboxylic group, only one pK_a value could be determined for these two compounds. The determination of the pK_a values of these compounds is quite straightforward, as described previously [11–14,19,20]. The pK_a values of nalidixic acid and flumequine determined are 6.00 and 6.35, respectively. These pK_a values were in good agreement with the literature values [3–5,7,8]. The results reflect that the pK_a values attributable to the ionization of the carboxylic moiety are around 6.00–6.35.

3.2.2. *Mobility equation involving two protolytic equilibria*

Two pK_a values were determined for 1-phenylpiperazine which involved two protonation/deprotonation equilibria. The variation of the electrophoretic mobility of this compound as a function of buffer pH (shown in Fig. 2) could be described y the following equation [2,10,16]:

$$\mu_{eff} = \frac{[H_3O^+]^2 \mu_{BH_2^{2+}} + K_{a1}[H_3O^+]\mu_{BH^+}}{[H_3O^+]^2 + K_{a1}[H_3O^+] + K_{a1}K_{a2}} \quad (2)$$

where μ_{BH^+} and $\mu_{BH_2^{2+}}$, respectively, are the electrophoretic mobilities of the first and second protonated species of the solute. The two pK_a values and two limiting mobilities of 1-phenylpiperazine were then determined by adjusting the trial values of these four parameters and by curve-fitting the experimental mobility data with the predicted mobility curve as a function of buffer pH through the utilization of Microcal Origin software until the best fit was obtained. For illustration, Fig. 3 shows the best fit of the mobility curve of 1-phenylpiperazine. As can be seen, good agreement between the predicted and observed mobility curves were obtained. The two pK_a values determined for 1-phenylpiperazine are 6.30 and 8.80 (see Table 1). These two pK_a values are qualitatively agreeable with those of piperazine reported in the literature, which are 5.33 and 9.73 [9]. The results clearly reveal that two protonation/deprotonation equilibria are involved in the piperazinyl moiety of a compound.

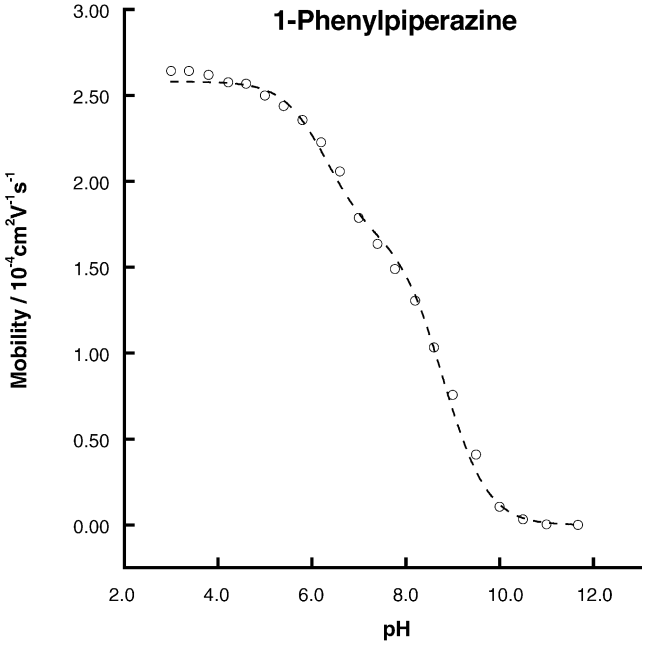

Fig. 3. The agreement between the predicted mobility curves (represented by dashed lines) and observed mobility curves (shown by data points) for 1-phenylpiperazine (◯).

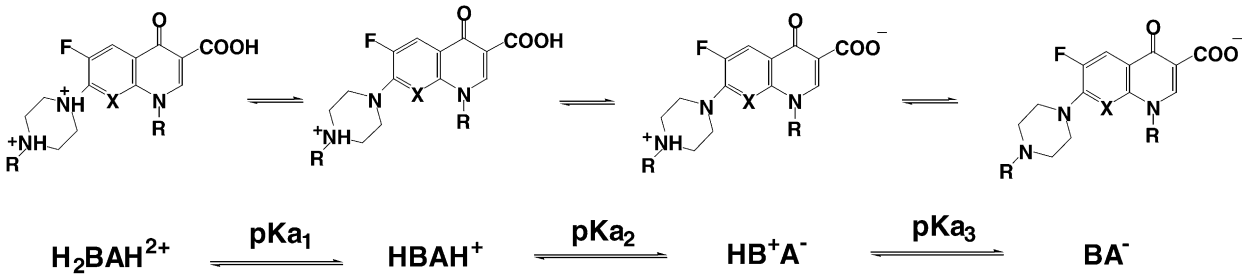

Fig. 4. Protonation/deprotonation equilibria of quinolones with a piperazinyl substituent.

3.2.3. Mobility equations involving three protolytic equilibria

It should be pointed out that the mobility equation derived previously by Barbosa et al. [3] is good for describing the variation of the electrophoretic mobility of quinolones involving two protonation/deprotonation equilibria, but not appropriate for describing the electrophoretic mobility of quinolones with a piperazinyl substituent. As two protonation/deprotonation equilibria are involved in piperazine [9] and in the piperazinyl moiety of compounds such as phenothiazines with a piperazinyl group [10], it is thought that three protonation/deprotonation equilibria should be involved for quinolones with a piperazinyl substituent.

Fig. 4 shows the schematic diagram of the protonation/deprotonation equilibria of quinolones with a piperazinyl substituent. As indicated, three charged species designated as H_2BAH^{2+}, $HBAH^+$ and BA^-, together with a zwitterionic species, designated as HB^+A^-, exist in the electrophoretic system. The effective electrophoretic mobility of each individual quinolone with a piperazinyl substituent is mainly contributed from two positively charged species and one negatively charged species, because zwitterionic species (HB^+A^-) is usually assumed to have zero net charge.

In the case that HB^+A^- possesses no net charge, the effective electrophoretic mobility of these quinolone compounds is described by the equation as derived for phenothiazines with a piperazinyl group at low pH [10]. This mobility equation can also be obtained from a generalized mobility equation involving multiprotolytic equilibria [16] and is given by:

$$\begin{aligned}\mu_{\text{eff}} &= \alpha_{H_2BAH^{2+}}\mu_{H_2BAH^{2+}} + \alpha_{HBAH^+}\mu_{HBAH^+} + \alpha_{BA^-}\mu_{BA^-} \\ &= \frac{[H_3O^+]^3\mu_{H_2BAH^{2+}} + K_{a1}[H_3O^+]^2\mu_{HBAH^+} + K_{a1}K_{a2}K_{a3}\mu_{BA^-}}{[H_3O^+]^3 + K_{a1}[H_3O^+]^2 + K_{a1}K_{a2}[H_3O^+] + K_{a1}K_{a2}K_{a3}}\end{aligned} \quad (3)$$

where μ and α represent the limiting electrophoretic mobility and mole fraction, respectively, of a particular charged species of quinolones and K_a represents the dissociation constants of the charged species. K_{a1}, K_{a2} and K_{a3} refer to the dissociation constants for the first deprotonation of the piperazinyl substitutent, the dissociation of the carboxylic group, and the second deprotonation of the piperazinyl substitutent, respectively, of the solutes, while $pK_{a1} < pK_{a2} < pK_{a3}$.

On the other hand, based on the fact that the amphoteric compounds such as 4-hydroxyphenylalanine are slightly charged at pH 7.0 [11], it may be reasonable to assume that HB^+A^- possesses a slightly negative charge at pH 7.0. Besides, no significant difference in the mobility curves of quinolones studied were observed with or without the addition of neutral marker in the electrophoretic system. Thus, the contribution of the mobility of HB^+A^- to the overall effective electrophoretic mobility of these quinolones is not completely negligible. This is caused by a stronger dissociation of the carboxyl group as compared to the protonation of the amino group. Thus, the mobility equation can be expressed as:

$$\begin{aligned}\mu_{\text{eff}} &= \alpha_{H_2BAH^{2+}}\mu_{H_2BAH^{2+}} + \alpha_{HBAH^+}\mu_{HBAH^+} + \alpha_{HB^+A^-}\mu_{HB^+A^-} + \alpha_{BA^-}\mu_{BA^-} \\ &= \frac{[H_3O^+]^3\mu_{H_2BAH^{2+}} + K_{a1}[H_3O^+]^2\mu_{HBAH^+} + K_{a1}K_{a2}[H_3O^+]\mu_{HB^+A^-} + K_{a1}K_{a2}K_{a3}\mu_{BA^-}}{[H_3O^+]^3 + K_{a1}[H_3O^+]^2 + K_{a1}K_{a2}[H_3O^+] + K_{a1}K_{a2}K_{a3}}\end{aligned} \quad (4)$$

Accordingly, the migration behavior of quinolones with a piperazinyl substitutent can then be predicted, once the pK_a values and limiting electrophoretic mobility are known.

Although the analysis of the mobility curves of quinolones with a piperazinyl substitutent according to Eq. (4) is not so straightforward because one of the inflection points of the mobility curve is obscurely observed, with the aid of computer simulation, the difficulty can be overcome and the inflection points can be determined for compounds with two or more consecutively close pK_a values. For instance, the mobility curve of tyrosine exhibits only one inflection point, but two pK_a values were determined at 8.94 and 9.99 [16]; only one inflection point appeared in the mobility curve of isophthalic acid, but two pK_a values were determined at 3.63 and 4.58 [2]. Hence, we believe that, with the aid of computer simulation and by consulting the pK_a values of compounds possessing a piperazinyl substituent, cloudiness of the determination of pK_a values can be clarified.

Table 2
The pK_a values and limiting mobility data of quinolones and piperazine derivatives evaluated according to Eq. (3)

Analytes	pK_a values			Limiting mobility[a]		
	pK_{a3}	pK_{a2}	pK_{a1}	μ_{BA}	μ_{HBAH^+}	$\mu_{H_2BAH^{2+}}$
Quinolones(with a piperazine substituent)						
Ofloxacin (1)	8.00 ± 0.03	5.85 ± 0.05	5.20 ± 0.08	−1.45 ± 0.01	1.00 ± 0.03	1.85 ± 0.01
Enoxacin (2)	8.30 ± 0.03	5.75 ± 0.06	5.15 ± 0.10	−1.60 ± 0.02	1.10 ± 0.03	2.00 ± 0.02
Norfloxacin (3)	8.10 ± 0.04	5.85 ± 0.08	5.10 ± 0.15	−1.55 ± 0.02	1.00 ± 0.03	1.95 ± 0.01
Lomefloxacin (4)	8.55 ± 0.04	5.80 ± 0.10	5.15 ± 0.15	−1.50 ± 0.01	0.90 ± 0.02	1.78 ± 0.01
Ciprofloxacin (5)	8.25 ± 0.05	5.75 ± 0.15	5.15 ± 0.20	−1.54 ± 0.02	0.95 ± 0.03	1.88 ± 0.02
Pipemidic acid (6)	8.35 ± 0.04	5.80 ± 0.10	5.25 ± 0.20	−1.63 ± 0.02	0.95 ± 0.03	1.95 ± 0.02

[a] Mobility in unit of /10^{-4} cm^2 V^{-1} s^{-1}.

The determination of the pK_a values of these quinolones requires the initial values of the pK_a and the limiting mobility of the three charged species, and a trial value for the mobility of the zwitterionic species as well, according to Eq. (4) or (3). The initial values of $\mu_{H_2BAH^{2+}}$ and μ_{BA^-} can be estimated from the mobility curve at pH < 3.5 and pH > 9.5, respectively. The initial value of μ_{HBAH^+} estimated is about the half of the initial value of $\mu_{H_2BAH^{2+}}$. In the case of HB^+A^- possessing zero net charge, the initial values of pK_{a3} can be estimated from the pH values corresponding to the half of the limiting mobility of the negatively charged species of each individual quinolone, which is about 8.0–8.5, but when HB^+A^- is slightly negatively charged, a little higher value is expected for the initial value of pK_{a3}. The pK_{a1} values are expected to fall in the pH range of 3.7–5.6 for quinolones with a piperazinyl substituent because the difference between the pK_{a1} and pK_{a3} values are very likely to fall in the range 2.5–4.4. This is based on the facts that the difference of the two pK_a values determined for 1-phenylpiperazine is 2.5 and those for piperazine and phenothiazines with a piperazinyl substitutent are in the range of 4.0–4.4 [9,10]. Moreover, the pK_a values of anilinium compounds also fall in the pH range 4.5–5.1 [9]. Furthermore, the two pK_a values of phenothiazines with a piperazinyl moiety reported are in the ranges of 3.60–3.86 and 7.90–8.15 [10]. Judging from the shape of the mobility curves of quinolones with a piperazinyl substitutent shown in Fig. 2, the expected range of the pK_{a1} value is further narrowed down to 4.7–5.6 because the inflection point for the determination of pK_{a1} is very unlikely to occur in the pH range of 3.0–4.7 or below 3.0. The pK_{a2} values are expected close to the pK_a values of nalidixic acid and flumequine. Hence, it is reasonable to set the initial value of pK_{a2} in the range of 5.7–6.5.

The three pK_a values and three or four limiting mobilities of each individual quinolone with a piperazinyl substitutent were then determined by adjusting the trial values of these six or seven parameters simultaneously and by curve-fitting the experimental mobility data with the predicted mobility curve as a function of buffer pH through the utilization of Microcal Origin software until the best fit was obtained. Tables 1 and 2 list the pK_a values and limiting mobilities evaluated for six quinolones with a piperazinyl substitutent, according to Eqs. (4) and (3), respectively, and those for two quinolones without a piperazinyl substituent, and 1-phenylpiperazine, together with the literature pK_a values of quinolones and piperazine.

In the case that HB^+A^- possesses zero net charge, the agreement between the predicted mobility curve based on Eq. (3) and the observed mobility curve in the pH range of

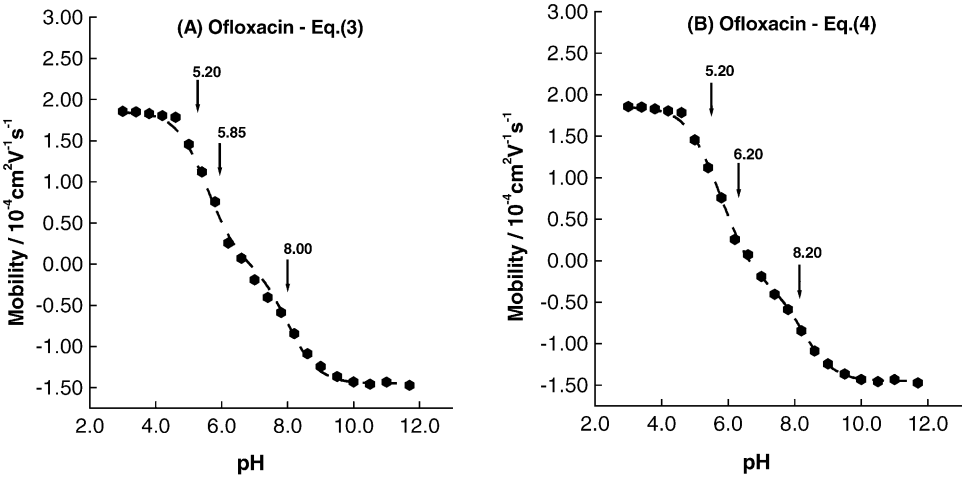

Fig. 5. The agreement between the predicted mobility curve according to (A) Eq. (3) and (B) Eq. (4) (represented by dashed lines) and observed mobility curve (shown by data points) for ofloxacin (⬢).

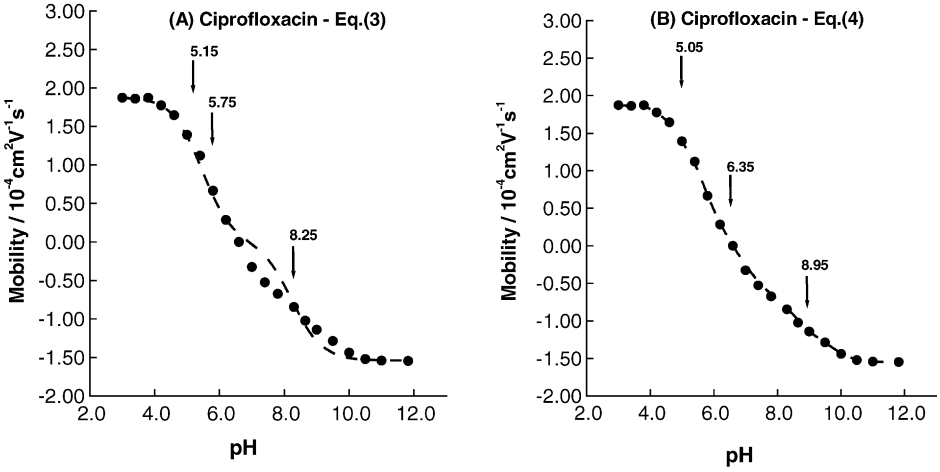

Fig. 6. The agreement between the predicted mobility curve according to (A) Eq. (3) and (B) Eq. (4) (represented by dashed lines) and observed mobility curve (shown by data points) for ciprofloxacin (●).

6.0–10.0 was poor for quinolones with a piperazinyl substituent, except for ofloxacin. The agreement between the predicted and observed mobility curve was fair, because the pK_{a3} value of ofloxacin evaluated was comparatively lower than those of the others. On the other hand, good agreement between the predicted mobility curves based on Eq. (4) and the observed mobility curves were obtained for all six quinolones when HB^+A^- possessing a slightly negative charge was considered. For illustration, Figs. 5 and 6 show the agreement between the predicted mobility curves based on Eqs. (3) and (4) and the observed mobility curves for ofloxacin and ciprofloxacin, respectively. As can be seen, the predicted mobility curves of ofloxacin and ciprofloxacin based on Eq. (4) agree very well with the observed mobility curves, especially in the pH range of 6.0–10.0. Evidently, the results obtained in this study clearly demonstrate that three protonation/deprotonation equilibria are involved for quinolones with a piperazinyl substituent and that Eq. (4) is more appropriate than Eq. (3) for describing the electrophoretic behavior of quinolones with a piperazinyl substituent. In other words, the results may suggest that the zwitterionic species (HB^+A^-) of quinolones with a piperazinyl substitutent are slightly negatively charged. In order to obtain further support, experiments for the determination of pK_a values of these quinolones by nuclear magnetic resonance are undertaken.

4. Conclusion

Three protonation/deprotonation equilibria, instead of two, are involved in quinolones with a piperazinyl substituent. With the aid of computer simulation, three pK_a values were determined by CE for quinolones with a piperazinyl substituent through the analysis of mobility curves presented as a function of buffer pH. The zwitterionic species of quinolones with a piperazinyl substituent is slightly negatively charged and the contribution of the zwitterionic species to the effective mobility may not be neglected. The pK_a values determined allow us to rationalize the influence of buffer pH on the electrophoretic behavior of these quinolones in CZE.

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