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Short Communication

Dissociation and complexation of the fluoroquinolone antimicrobials — an update

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Introduction

The fluoroquinolones are an important class of antimicrobials agents (Fig. 1) whose behaviour *in vivo* is significantly influenced by their physicochemical properties, in particular their degree of ionization [1–7] and their capacity to chelate metal ions. Whereas the fluoroquinolones have several potentially ionizable functional groups only two, the carboxylic acid and piperazine, are of pharmaceutical importance.

Ionization

The ionization of the fluoroquinolones can be conveniently described by the scheme shown in Fig. 2 and there is extensive literature showing that the values for $pK_{a,1}$ and $pK_{a,2}$ are approximately 5.7–6.2 and 7.9–8.9, respectively (Table 1). The value of $pK_{a,1}$ for the fluoroquinolones is higher than is generally observed for carboxylic acids. This decrease in acidity has been attributed to intramolecular hydrogen-bond formation between the carboxylic acid and the neighboring keto function resulting in stabilization of protonated species (Fig. 3). The value of $pK_{a,1}$, corresponding to dissociation of the carboxylic acid appears to be relatively insensitive to substitution on the quinolone. On the other hand, the value of $pK_{a,2}$ is sensitive to substitution on the piperazine ring [1]. In addition to the presence of one or more fluorine substituents, the presence of a piperazine ring differentiates the fluoroquinolones from their predecessor, nalidixic acid, which may be treated essentially as a weak acid within the pH ranges of pharmaceutical or physiological importance.

Structural effects

Recently, Lee *et al.* [8] have reported values for $pK_{a,1}$, corresponding to the dissociation of the carboxylic acid of norfloxacin, ofloxacin, nalidixic acid and enoxacin (Figs 1 and 2) of between 0.7 and 1.0, which are about 5 pH units lower than those reported previously in the literature [1–7] (Table 1). Also, Lee *et al.* have incorrectly reported the values of $pK_{a,2}$ of ciprofloxacin and enoxacin as 7.41 and 6.71, respectively, which compare with previous reports of 8.66–8.80 and 8.50–8.69 (Table 1). Furthermore, Lee *et al.* [8] report a $pK_{a,2}$ value of 6.36 for nalidixic acid, despite the absence of a piperazine ring.

Lee *et al.* [8] attribute the discrepancies between their data and the literature to differences in analytical methodology. However, a more likely explanation for those discrepancies is the incorrect assignment of the ionizable functional groups. Whereas the value of $pK_{a,1}$ for ciprofloxacin is reported correctly and is consistent with dissociation of a carboxylic acid stabilized by intramolecular hydrogen bonding, the value of $pK_{a,1}$ between 0.7 and 1.0 is incorrectly attributed to the dissociation of the carboxylic acid group in nalidixic acid,

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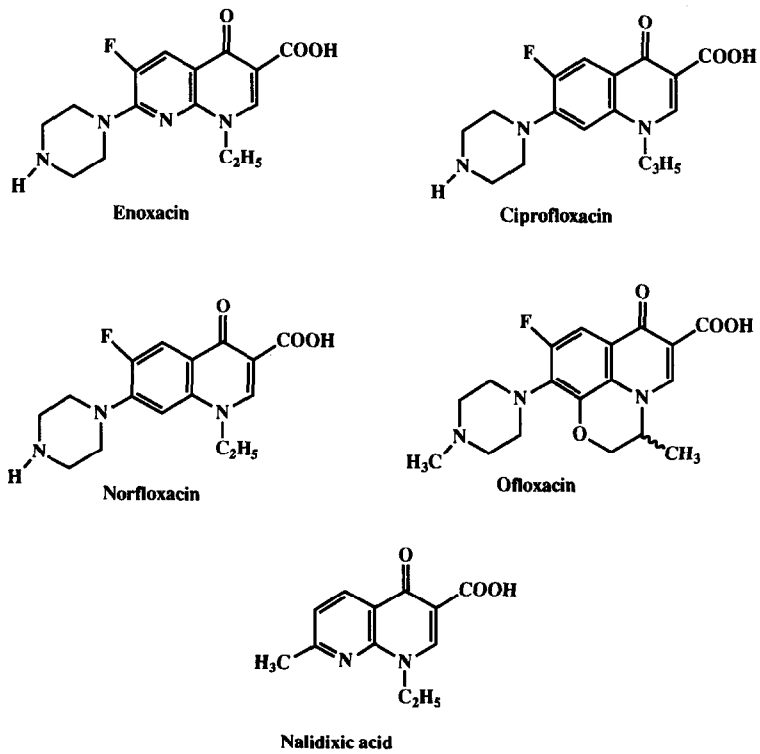

Figure 1
Structures of selected fluoroquinolone antimicrobials.

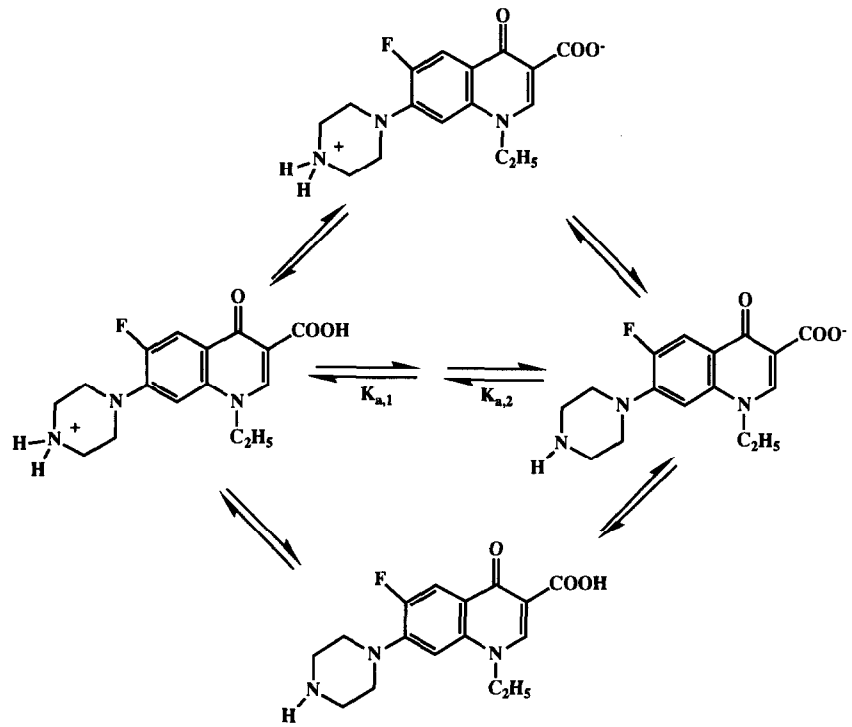

Figure 2
Ionic dissociation of the fluoroquinolone, norfloxacin.

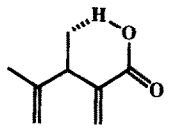

Figure 3
Intramolecular hydrogen bonding of the carboxylic acid and the adjacent keto function.

enoxacin, norfloxacin and ofloxacin. Staroscik and Sulkowska [5] have reported that the pK_a value for the naphthyridine nitrogen in nalidixic acid is 0.94. Thus it would appear the $pK_{a,1}$ values reported by Lee *et al.* [8] for nalidixic acid, enoxacin, norfloxacin and ofloxacin would be more appropriately assigned to one of the heterocyclic nitrogen atoms rather than to the carboxylic acids. It follows, then, that the $pK_{a,2}$ values of 6.71 for enoxacin and 6.36 for nalidixic acid are more appropriately assigned to the carboxylic acid (i.e. $pK_{a,1}$ in Fig. 2). They have correctly assigned the $pK_{a,1}$ value of 6.14 to the carboxylic acid of ciprofloxacin and that value is in good agreement with the literature. However, the reason for the large discrepancy between the ciprofloxacin $pK_{a,2}$ value of 7.41 reported by Lee et al. [8] and the values reported (Table 1) earlier (8.74–8.80) is unclear.

Linear free energy relationships

The use of a Hammett plot [8] to relate the dissociation constants for the carboxylic acids to structure would appear appropriate. However, the incorrect assignment of the $pK_{a,1}$ values of enoxacin, norfloxacin, nalidixic acid and ofloxacin would seem to bring that relationship [8] into question. In addition, the conclusion [8] that the dissociation of the fluoroquinolones is influenced by substitution at the N-1 position is unsubstantiated, because enoxacin, norfloxacin and nalidixic acid each have an ethyl group at that position. Also it seems unlikely that the cyclopropyl group would have a substantially different electronic effect than an ethyl group. Of the five quinolones studied by Lee *et al.*, only the C-8 oxygen in ofloxacin could be expected to exert an electron-withdrawing effect that would not be present in the other four compounds. Each of the fluoroquinolones contain a fluorine at the C-6 position, which could exert an inductive electron-withdrawing effect and thereby modulate the dissociation of the carboxylic acid. However, the distance between the C-6 fluorine atom and the carboxylic acid appears to be too large to influence the value of $pK_{a,1}$. This conclusion is supported by the fact that the $pK_{a,1}$ value for nalidixic acid, which lacks a fluorine at C-6, is similar to the values reported for the fluoroquinolones.

The use of the Hammett equation [8] to describe the influence of substitution on the value of $pK_{a,2}$ is inappropriate because the

Table 1
Apparent macroscopic pK_a values and isoelectric points (pI) of selected quinolone antimicrobials

	$pK_{a,1}$		$pK_{a,2}$		pI	
Quinolone	Lee *et al.* [8]	Literature	Lee *et al.* [8]	Literature	Lee *et al.* [8]	Literature*
Ciprofloxacin	6.14	6.09 [1] 6.15 [2] 6.00 [3]	7.41	8.74 [1] 8.66 [2] 8.80 [3]	6.78	7.41
Enoxacin	0.71	6.31 [1] 6.00 [4]	6.71	8.69 [1] 8.50 [4]	3.71	7.38
Nalidixic acid	0.95	(0.94)† 5.95 [1] 6.02 [5] 6.12 [5]	6.36	n.a.‡	6.36	n.a.‡
Norfloxacin	0.74	6.30 [1] 6.54 [2] 6.40 [3] 6.20 [6] 6.22 [7]	8.26	8.38 [1] 8.50 [2] 8.70 [3] 8.70 [6] 8.51 [7]	4.50	7.44
Ofloxacin	0.90	6.05 [1] 5.70 [3]	7.88	8.22 [1] 7.90 [3]	4.39	6.97

*Calculated from equation 1 using average of literature values.
†Attributed to the protonation of the heterocyclic N-8 nitrogen.
‡n.a. = not applicable.

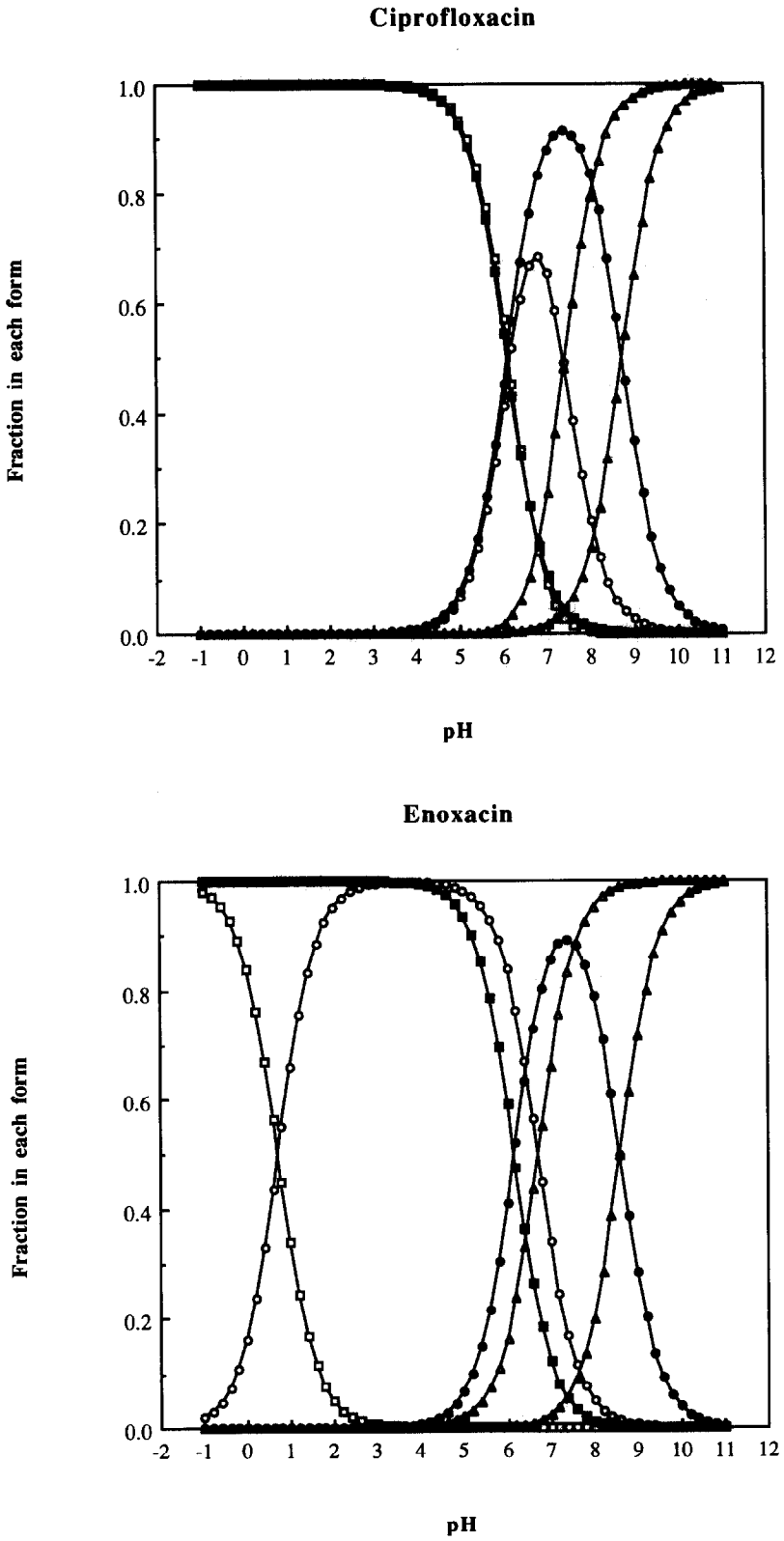

Figure 4
Fractional distribution diagrams for (a) ciprofloxacin, (b) enoxacin, (c) norfloxacin and (d) ofloxacin. The open symbols represent the data calculated from Lee *et al.* [8]. The closed symbols represent the data calculated from the averaged literature pK_a values (Table 1). The squares represent the cationic species (H_2Q^+), the circles represent the total of the zwitterionic (HQ^+) and the neutral (HQ^o) species, and triangles represent the anionic species (Q^-).

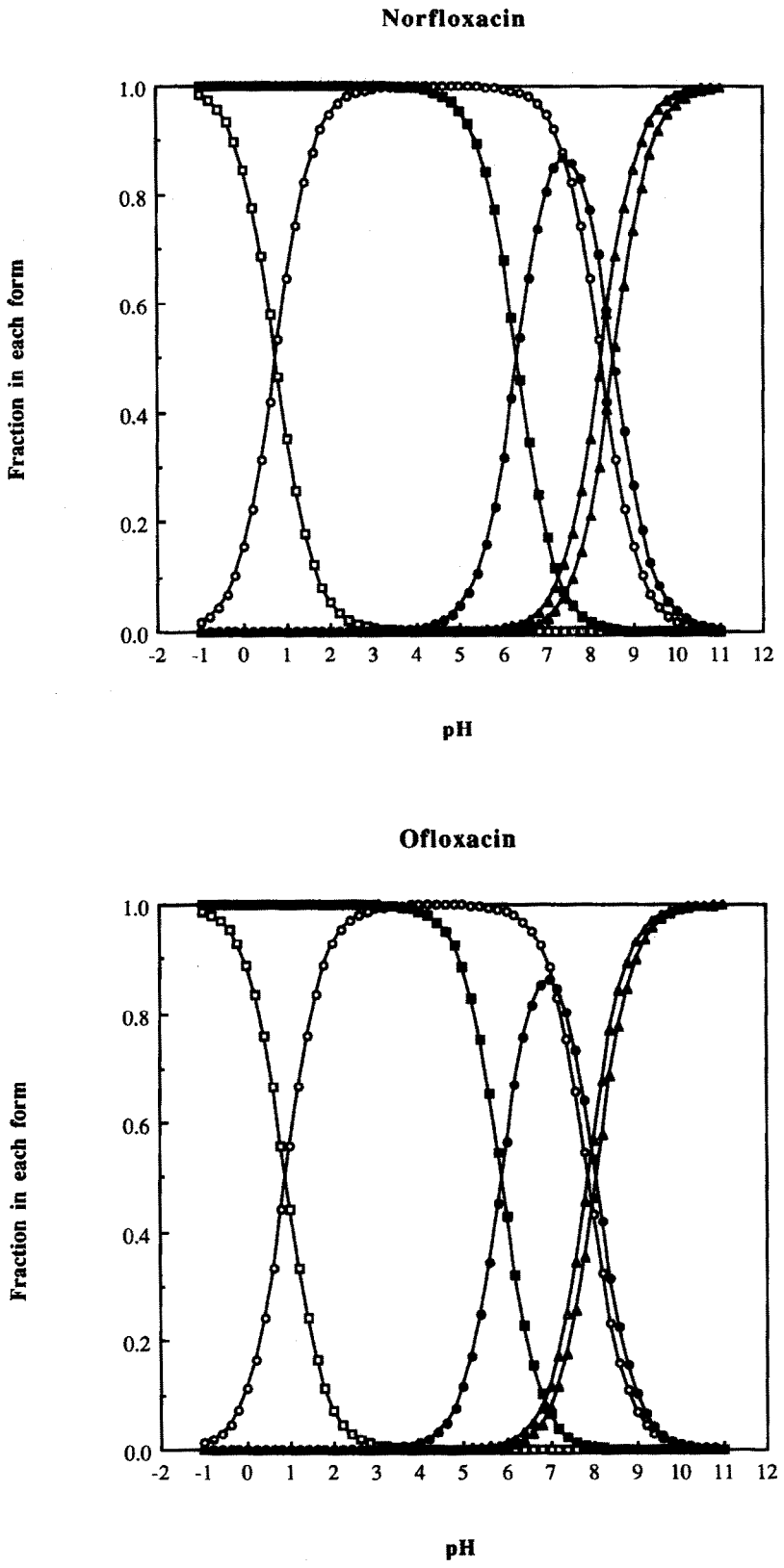

Figure 4
Continued.

piperazinyl nitrogen involved is not associated with a conjugated π-electron system. The only demonstrated effect of substitution on the protonation of the piperazine nitrogen comes from the work of Ross and Riley [9] who showed that introduction of a methyl group at the 4′-N position lowers the value of $pK_{a,2}$ by about 0.5–1.0 pH units, due to a steric effect that destabilizes the proton. This explains the lower $pK_{a,2}$ value of ofloxacin (Table 1) compared with the 4′-unsubstituted analogues.

Metal-ion Complexation

Much of the early work on the metal ion complexation of the quinolones was done by Ruzicka *et al.* [10] and Vincent *et al.* [11], who reported the formation of 2:1 complexes between nalidixic acid and Fe^{3+} in aqueous solution. In contrast, Behrens and Mendoza-Diaz [12] synthesized 3:1 complexes between nalidixic acid and Fe^{3+}. Issopoulos [13] reported 2:1 complexes between norfloxacin and Fe^{3+} and work in this laboratory [14] has demonstrated 2:1 complexes with lomefloxacin and Fe^{3+}.

Lee *et al.* [8] have described 1:1 complexes between the fluoroquinolones and Fe^{3+}; however, their non-linear Scatchard plots are indicative of higher order complexes. Furthermore, the different methods of determination of the association constant used in that study [8] gave results that differed by 1–3 orders of magnitude for a given fluoroquinolone. This is further evidence of the incorrect stoichiometry assumed by Lee *et al.* [8], because work conducted here [14, 15] and elsewhere [10–13, 16, 17] has shown that metal ion complexation constants for all the fluoroquinolone studied to date differ by less than two orders of magnitude.

Binding of metal ions at the 3,4-β-diketone residue of the fluoroquinolones is well documented [10–17] and is supported by several studies including NMR studies, published in this Journal, [15] and elsewhere [16, 17] demonstrating the complexation of norfloxacin, lomefloxacin and nalidixic acid with aluminum and magnesium. However, attempts by this group to isolate metal-ion complexes of any of the fluoroquinolones from aqueous solution have been unsuccessful because the complexes were always more soluble than the uncomplexed material. The small changes in the IR spectra reported by Lee *et al.* [8] are more consistent with a physical mixture of the fluoroquinolones and Fe^{3+} salts rather than the fluoroquinolone–metal ion chelate.

Conclusions

The values of $pK_{a,1}$ and $pK_{a,2}$ are important because they determine the degree of ionization of the fluoroquinolones, which exist predominantly in the zwitterionic form at physiological pH, since the isoelectric point (pI) is given:

$$pI = 0.5(pK_{a,1} + pK_{a,2}) \quad (1)$$

Lee *et al.* correctly indicate that an appreciation of the fractional distribution of the Bjerrum species of the fluoroquinolones is important in the understanding of their biological properties. However, the discrepancies between the dissociation constants reported by Lee *et al.* [8] and those reported previously in the literature lead to conflicting values of the isoelectric point (equation 1, Table 1). Furthermore, Fig. 4 shows the substantial differences in the fractional distribution of the Bjerrum species calculated using the pK_a values reported by Lee *et al.* compared with those calculated using previous values in the literature. Their conclusion that the zwitterionic forms of enoxacin, norfloxacin and ofloxacin predominate at pH 3.80 is clearly incorrect. Furthermore, their conclusion is not supported by studies conducted in this laboratory, which showed minima in the pH-solubility profiles [1] and maxima in the pH-partition profiles [18] around pH 7 for all the fluoroquinolones studied.

There is a substantial body of literature [10–17] to support the hypothesis that the quinolone antimicrobials chelate metal ions at the 3,4-β-diketone. In general chelation of metal ions increases the aqueous solubilities and decreases the partition coefficients of the fluoroquinolones. Those effects, in turn, appear to lead to decreased oral bioavailability and decreased penetration of the fluoroquinolones if they are coadministered with antacids, iron supplements or other metal-ion containing products. However, for a given metal ion the association constant is relatively insensitive to changes in fluoroquinolone structure. In contrast, the association constants of the quinolones is significantly dependent on the nature of the metal ion, generally increasing in the

order: $Ca^{2+}<Mg^{+}<Br^{2+}<Fe^{3+}<Al^{3+}$ [9, 14, 15, 18].

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