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Short communication

Protonation equilibrium and lipophilicity of moxifloxacin

Marie-Hélène Langlois[a], Martine Montagut[a], Jean-Pierre Dubost[a,*], Jean Grellet[b], Marie-Claude Saux[b]

[a] *Laboratory of Analytical Chemistry, EA 2962, Bordeaux Il University, Bordeaux, France*
[b] *Laboratory of Pharmacokinetics and Clinical Pharmacy, EA 525, Bordeaux II University, Bordeaux, France*

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Abstract

This study was performed to characterise the protonation equilibrium at the molecular level and pH-dependent lipophilicity of moxifloxacin. After determining macro- and micro-constants, distribution features of four microspecies in aqueous phase were assessed. The apparent partition coefficient versus pH profile of moxifloxacin showed a parabolic curve in *n*-octanol/buffer system which reached near *pI*. The true partition coefficient was calculated from the log P_{app} and microconstants values.

Keywords: Moxifloxacin; Octanol/buffer partition coefficients; Macroconstants; Microconstants

1. Introduction

Moxifloxacin is a fourth-generation fluoroquinolone with potential efficacy in the treatment of various community-acquired and nosocomial infections [1]. Moreover, this compound appears to cover bacterial resistance to second- and third-generation fluoroquinolones [2–6]. It has the 6-fluorine substituent generally present in the currently used quinolones and contains a cyclopropyl group at position 1, a diazabicyclononyl moiety at position 7 and a methoxy group at position 8 (Fig. 1).

However, very little research has been carried out on its physicochemical parameters such as pK_a and partition coefficient, which exert a key role in determining its absorption, transport and receptor binding at a molecular level [7]. Therefore, knowledge of the physical and chemical properties of this drug may be essential for the interpretation of structure–activity relationships.

In this study, we determined the acid–base properties of moxifloxacin (amphoteric molecule) in terms of macro- and micro-dissociation constants by means of potentiometry plus spectrophotometry, and studied its pH-behavior in terms of the apparent partition coefficient in an *n*-octanol/buffer system.

2. Experimental

2.1. Reagents

Moxifloxacin hydrochloride was generously provided by the Laboratory of Pharmacokinetics and Clinical Pharmacy.

A 1-octanol was purchased from Aldrich and all other reagents were of at least analytical grade.

2.2. Measurement of protonation equilibrium

Molecules with two proton-binding sites such as moxifloxacin (basic group in the 7-position and acid group in the 3-position) exist in four microspecies in aqueous solution, namely positive (H_2Q^+), zwitterionic ($HQ^{\pm}$), neutral (HQ^o) and negative (Q^-) microforms at the molecular level [7–9]. The scheme of the protonation equilibrium between the four microspecies as well as the relevant macro- and microconstants is shown in Fig. 2.

* Corresponding author. Tel.: +33 5 57574683; fax: +33 5 57574687.
E-mail address: jean-pierre.dubost@u-bordeaux2.fr (J.-P. Dubost).

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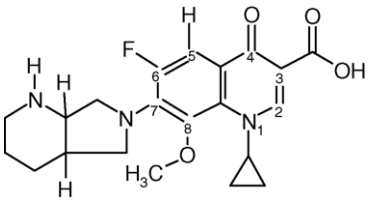

Fig. 1. Chemical structure of moxifloxacin.

Macroconstants were determined by potentiometry using a radiometer pHM201 pH meter equipped with a glass combinated electrode radiometer pHC3005. The electrode was calibrated with standard buffer solutions (pH 4.00–7.00).

Aliquots of 50 ml of 0.5 mM moxifloxacin hydrochloride in 10^{-3} M HCl were titrated by 10^{-2} M NaOH. Aliquots of 50 ml of 0.5 mM moxifloxacin hydrochloride in 1.5×10^{-3} M NaOH were titrated by 10^{-2} M HCl. All measurements were made at 0.15 M ionic strength, using NaCl as the auxiliary electrolyte. During neutralisation the samples were continuously stirred with a magnetic stirrer; pH values were measured for each 0.1 ml affusion.

Microconstants were determined by spectrophotometry using a Jasco V530 spectrophotometer. This method is based on the fact that the fluoroquinolone spectrum is independent of the protonation state of the diazabicyclononyl moiety, but it is heavily influenced by that of carboxylate. Thus, the degree of protonation at the carboxylate group can be selectively monitored by spectrophotometry.

Two aliquots of 30 μM moxifloxacin hydrochloride solutions were prepared in either 10^{-3} M HCl or 10^{-3} M NaOH with a total ionic strength of 0.15 M. A set of solutions were then prepared by mixing appropriate volumes of acid and base stock solutions. The spectrum of each solution was immediately recorded in the wavelength range of 200–400 nm.

2.3. Determination of apparent partition coefficient

The apparent partition coefficients in 1-octanol/buffer systems were measured using the shake-flask technique [10] at room temperature. The organic and aqueous phases were mutually saturated. Moxifloxacin hydrochloride was dissolved in aqueous buffer solutions (phosphate, borate) [11] with NaCl 0.1 M; the solutions were shaked with 1-octanol for 1 h by which the partition equilibrium was reached. The phase ratio (V_W/V_O) varied depending on the expected log P_{app} value. After equilibration, the 1-octanol phases were removed with a Pasteur pipette and then the aqueous phases were centrifuged at $700 \times g$. The concentration of the solute was determined in the aqueous phases by UV spectrophotometry at the λ_{max} of compound.

3. Results and discussion

3.1. Determination of macro-dissociation constants

Macroconstants quantitate the carboxylate and amine basicity of the molecule. From Fig. 2, we can express macro-

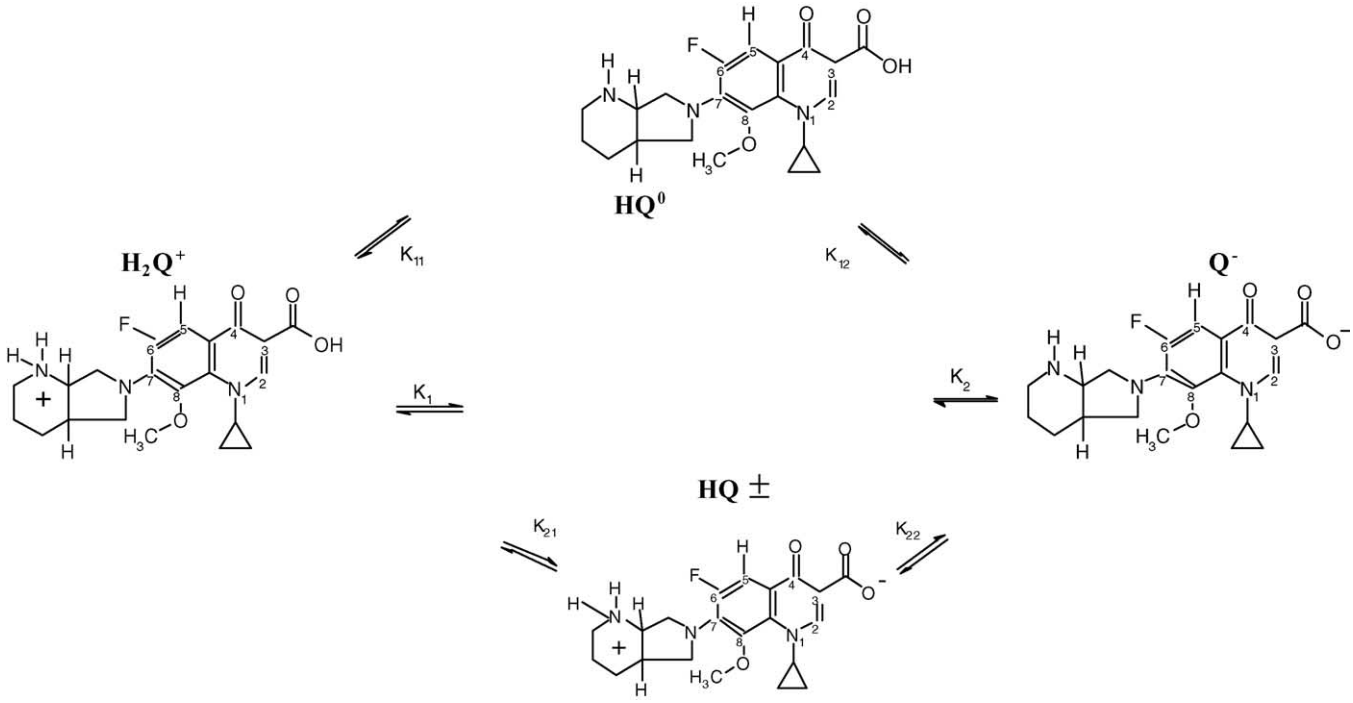

Fig. 2. Protonation equilibrium of moxifloxacin.

scopic constants K_1 and K_2 as:

$$K_1 = \frac{\{[HQ^{\pm}] + [HQ^{o}]\}[H^+]}{[H_2Q^+]} \quad (1)$$

$$K_2 = \frac{[Q^-][H^+]}{[HQ^{\pm}] + [HQ^{o}]} \quad (2)$$

where $\{[HQ^{\pm}] + [HQ^{o}]\}$ denotes the total concentration of zwitterion and uncharged species, which are indistinguishable by acid–base titration.

The pK_1 value of moxifloxacin (6.25 ± 0.02) shows that this compound is a weaker acid like ciprofloxacin [12]. The intra-molecular hydrogen-bond formation between the carboxyl and keto groups in the quinoline ring contributes to lowered acidic character for fluoroquinolones, because of improved stability of the protonated form of the carboxyl group.

The pK_2 value of moxifloxacin (9.29 ± 0.04) is similar to values previously reported for secondary amine fluoroquinolones [7,14].

3.2. Evaluation of micro-dissociation constants

The four microconstants can be expressed with microspecies concentrations as follows:

$$k_{11} = \frac{[HQ^{o}][H^+]}{[H_2Q^+]} \quad (3)$$

$$k_{12} = \frac{[Q^-][H^+]}{[HQ^{o}]} \quad (4)$$

$$k_{21} = \frac{[HQ^{\pm}][H^+]}{[H_2Q^+]} \quad (5)$$

$$k_{22} = \frac{[Q^-][H^+]}{[HQ^{\pm}]} \quad (6)$$

Relations between the micro- and macro-constants have been reported [13]:

$$K_1 = k_{11} + k_{21} \quad (7)$$

$$\frac{1}{K_2} = \frac{1}{k_{12}} + \frac{1}{k_{22}} \quad (8)$$

$$\beta = K_1K_2 = k_{11}k_{12} = k_{21}k_{22} \quad (9)$$

The determination of one of the microconstants is possible by molecular absorption spectroscopy because the acidic group of moxifloxacin has an absorption spectrum morphologically distinguishable from its conjugate base (Fig. 3).

So, the fraction of protonation on the carboxylate group at a given pH, α_{coo-}, can be calculated from spectrophotometric absorbance data as follows:

$$\alpha_{COO^-} = \frac{A_{pH} - A_{COOH}}{A_{COO^-} - A_{COOH}} \quad (10)$$

where A_{pH} is the absorbance at a given pH and A_{COO^-} and A_{COOH} are the absorbances at extremely basic and acid pH values.

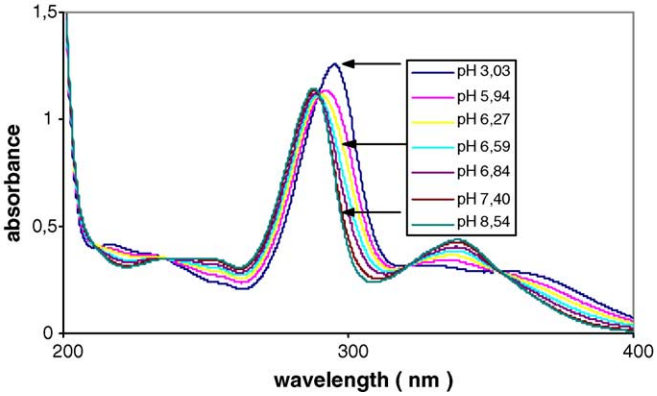

Fig. 3. pH-dependent UV absorption spectra for moxifloxacin.

Using relationships between α_{COO^-} and microspecies concentrations [12], as well as the equilibrium constants in Eqs. (1)–(9), the microconstant k_{21} can be expressed as follows:

$$\alpha_{COO^-} = \frac{[Q^-] + [HQ^{\pm}]}{[Q^-] + [HQ^{\pm}] + [HQ^{o}] + [H_2Q^+]} \quad (11)$$

$$\alpha_{COO^-} = \frac{K_{21}/[H^+] + \beta/[H^+]^2}{1 + K_1/[H^+] + \beta/[H^+]^2} \quad (12)$$

$$K_{21} = \alpha_{COO^-}\{K_1 + [H^+]\} - \{1 - \alpha_{COO^-}\}\frac{\beta}{[H^+]} \quad (13)$$

Once k_{21} determined from Eq. (13), the other microconstants are obtained from Eqs. (7)–(9).

The experimental values of protonation constants are summarized in Table 1.

The relative concentration for all microspecies in solution can be calculated with the following equations:

$$f[Q^-] = \frac{\beta}{\beta + K_1[H^+] + [H^+]^2} \quad (14)$$

$$f[HQ^{\pm}] = \frac{k_{21}[H^+]}{\beta + K_1[H^+] + [H^+]^2} \quad (15)$$

$$f[HQ^{o}] = \frac{k_{11}[H^+]}{\beta + K_1[H^+] + [H^+]^2} \quad (16)$$

$$f[H_2Q^+] = \frac{[H^+]^2}{\beta + K_1[H^+] + [H^+]^2} \quad (17)$$

Distribution diagram of the four microspecies of moxifloxacin is shown in Fig. 4.

This distribution diagram shows the predominance of neutral and zwitterion forms at physiological blood pH, whereas

Table 1
Protonation constants of moxifloxacin

Macroconstants	Microconstants	
$\log k_1 = -6.25$	$\log k_{11} = -7.46$	$\log k_{12} = -8.08$
$\log k_2 = -9.29$	$\log k_{21} = -6.29$	$\log k_{22} = -9.25$

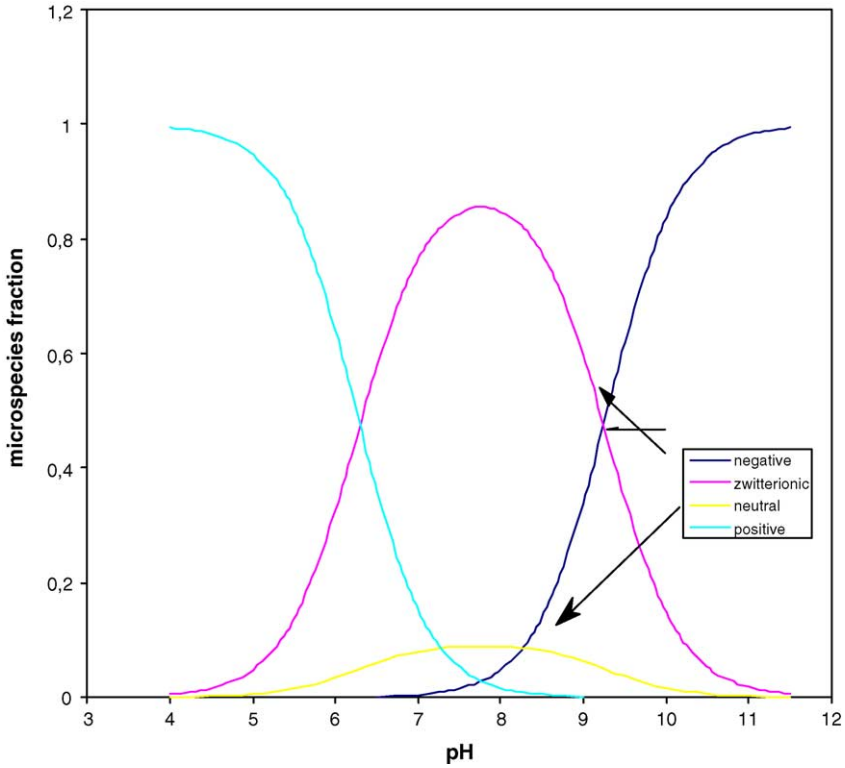

Fig. 4. The microspeciation diagram of moxifloxacin.

the other microspecies are minor components. Conversely positive and negative forms predominated at acid and basic values, respectively. It should be observed that a constant ratio (10.4%) exists between neutral and zwitterionic species in the pH range.

It has been found that this ratio differs considerably among quinolone antibacterials heavily influencing their lipophilicity and thus determining their ability to reach the interior of bacteria [7,14].

Moreover, microspeciation may explain the differences in the biological absorption of fluoroquinolones depending on the neutral-form concentration at blood pH. It can also be used for the determination of the true partition coefficient [15–16]:

$$\log P = \log P_{\text{app}} + \log\left(1 + \frac{k_{21}}{k_{11}} + \frac{k_{12}}{[\text{H}^+]} + \frac{[\text{H}^+]}{k_{11}}\right) \quad (18)$$

3.3. pH-dependence of the apparent partition coefficient

Results of QSAR studies indicate that hydrophobic, electronic and steric parameters of the fluoroquinolones play equally important roles in their biological activity. While receptor binding (interaction with the DNA–gyrase enzyme of the bacteria) is governed by the electronic and steric properties of N1, C6, C7 and C8 substituents, the lipophilicity of compounds seems to determine their penetration into the bacterial cell and affects the intestinal absorption and membrane permeability [17–19]. Moreover, this latter physicochemical property exerts an influence on the absorption, distribution, storage and elimination of fluoroquinolone drugs.

Fluoroquinolones like amphoteric molecules are ionised over the range of pH values. In the present study, we investigated pH-dependence in the partition behavior using a 1-octanol/buffer system. The apparent partition coefficient is described by total concentrations of organic phase divided by those of aqueous phases and calculated according to equation:

$$P_{\text{app}} = \frac{A_{\text{i}} - A_{\text{f}}}{A_{\text{f}}} \times \frac{V_{\text{w}}}{V_{\text{o}}} \quad (19)$$

where A_{i} and A_{f} represent the absorbance of moxifloxacin hydrochloride in the aqueous phase before and after partitioning, respectively. Each $\log P_{\text{app}}$ value is an average of five parallel measurements. The apparent partition coefficients of moxifloxacin hydrochloride determined at different pH values are summarized in Table 2.

Table 2
Apparent log *P* values of moxifloxacin at different pH values

pH	$\log P_{\text{app}}$
5.9	−0.689 ± 0.017
6.4	−0.508 ± 0.007
7.4	−0.280 ± 0.011
8.0	−0.237 ± 0.011
8.7	−0.352 ± 0.002

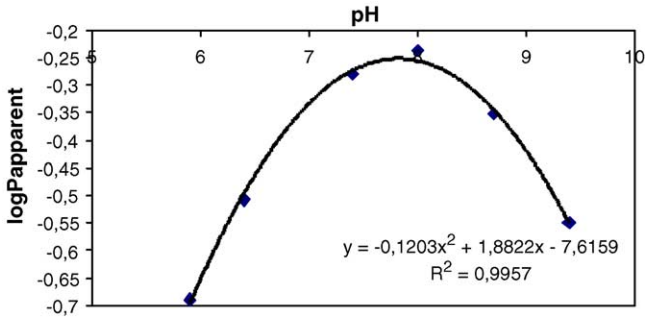

Fig. 5. pH-partitioning profile of moxifloxacin.

The pH-partition profile of this compound has a parabolic shape, which reflects the maximum lipophilicity of moxifloxacin at its isoelectric point (Fig. 5).

If we look at the curves for the pH-partition behavior and the microspeciation of moxifloxacin, it becomes evident that plots of the concentration of zwitterionic and non-ionic forms as a function of pH show similar maximum shapes within the same pH range. Maximum partitioning into the lipid phase occurs when an uncharged species is present in a commensurable concentration relative to the zwitterionic form.

The true partition coefficient can be calculated according to Eq. (18). By comparing the $\log P$ value (0.832 ± 0.057) with values of fluoroquinolones previously published, moxifloxacin can be considered to be a lipophilic compound [20].

This information could be used in QSAR studies where $\log P$ would be used to evaluate the biological activity of fluoroquinolones and also in the prediction of their ability to permeate the external barriers of microbes.

References

[1] J.M. Blondeau, Clin. Therm. 21 (1999) 3–40.
[2] C.J. Soussy, Med. Mal. Infect. 33 (2003) 125–133.
[3] E. Bergogne-Berezin, Antibiotiques (2002) 57–64.
[4] J. Behra-Miellat, L. Dubreuil, L. Calvet, H. Marchandin, Med. Mal. Infect. 33 (2003) 27–34.
[5] J.C. Rodriguez, M. Ruiz, M. Lopez, G. Royo, Int. J. Antimicrob. Agents 20 (2002) 464–467.
[6] J. Behra-Miellat, L. Dubreuil, E. Jumas-Bilak, Int. J. Antimicrob. Agents 20 (2002) 366–374.
[7] K. Takacs-Novak, B. Noszal, I. Hermecz, G. Kereszturi, B. Podanyi, G. Szasz, J. Pharm. Sci. 79 (1990) 1023–1028.
[8] Lee Dong-Sun, Han Hee-Jung, Kun Kim, Park Won-Bong, Cho Jung-Kil, Jae-Hyun Kim, J. Pharm. Biomed. Anal. 12 (1994) 157–164.
[9] S. Riegelman, L.A. Strait, E.Z. Fisher, J. Pharm. Sci. 51 (1962) 129–133.
[10] W.P. Purcell, G.E. Bass, J.M. Clayton, Strategy of Drug Design, in: A Molecular Guide to Biological Activity, App.I. Experimental Determination of Partition Coefficients, Wiley, New York, 1973, pp. 126–142.
[11] Pharmacopée Européenne, fourth ed., 2002, pp. 97–101.
[12] J. Hernandez-Borell, M.T. Montero, J. Chem. Educ. 74 (1997) 1311–1314.
[13] J.T. Edsall, J. Wyman, Biophysical Chemistry, vol. 1, Academic Press, New York, 1958, pp. 65–66 (Chapters 8–10).
[14] D.L. Ross, S.K. Elkinton, C.M. Riley, Int. J. Pharm. 88 (1992) 379–389.
[15] M.T. Montero, J. Freixas, J. Hernandez-Borrell, Int. J. Pharm. 149 (1997) 161–170.
[16] K. Takacks-Novak, M. Jozan, G. Szasz, Int. J. Pharm. 113 (1995) 47–55.
[17] T. Fujita, The Role of QSAR in Drug Design, Academic Press, New York, 1984, pp. 19–33.
[18] G. Stein, Pharmacotherapy 8 (1988) 301–314.
[19] M. Ohta, H. Koga, J. Med. Chem. 34 (1991) 131–139.
[20] K. Takacs-Novak, M. Jozan, I. Hermecz, G. Szasz, Int. J. Pharm. 79 (1992) 89–96.