Protonation Equilibria and Lipophilicity of Sarafloxacin

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The main objective of this work was to quantify the lipophilicity of sarafloxacin in the form of the apparent and true partition coefficient in an *n*-octanol/buffer system. For that purpose the ionization constants were determined by a spectrophotometric method, and the distribution profiles of microspecies in water as a function of pH for this fluoroquinolone were established. The maximum concentration of the neutral species occurs at the sarafloxacin isoelectric point, pH = 5.5. The apparent partition coefficient in the *n*-octanol/buffer system versus the pH profile of sarafloxacin displayed a parabolic behavior that reached a maximum near the pH of the isoelectric point. The true partition coefficient was calculated from the log P_{app} and microconstant values and has the value of 0.846 ± 0.021.

Introduction

Lipophilicity, usually expressed by the octanol−water partition coefficient (log *P*), constitutes a physicochemical property of paramount importance in medicinal chemistry since it plays an essential role in absorption, distribution, metabolism, and elimination characteristics of drugs while also affecting their pharmacodynamic and toxicological profile.[1] The majority of the structure−activity relationships developed and available uses lipophilicity as described by the octanol−water partition. The dominant role of octanol−water partition coefficients in the pharmacokinetic processes, as well as in ligand−macromolecule interactions, triggered further research on the development of reliable methods for their experimental assessment as well as of suitable calculation methods.[1−3] Despite all of the efforts, literature reviews can still yield log *P* values that differ by more than one order of magnitude for some compounds.[4,5]

Lipophilicity, however, is not merely a useful parameter in drug design. Being the outcome of preferential interactions of the drug molecules with more than one component, it encodes valuable structural information. However, ionization dramatically alters all types of intermolecular interactions involving the charged center, limiting the validity of the rules established for neutral compounds. In particular, amphoteric fluoroquinolones contain two proton-binding sites with similar basicity (a carboxyl and a secondary amine group)[6] which are responsible for the charge of a molecule in solution at a particular pH, and thus the log *P* value for ionizable compounds is pH-dependent.

This work focuses on a study of the lipophilicity of sarafloxacin, a fluoroquinolone used for veterinarian purposes. In particular, the effect of the 4-fluorophenyl group in the N_1 position will be evaluated and the results compared with those for other fluoroquinolones. In aqueous solution four microspecies exist,[6,7] as can be observed in Figure 1. The definition of the four microprotonation constants, k_{11}, k_{12}, k_{21}, and k_{22}, the two macroprotonation constants, K_1 and K_2, as well as the relationship between them are well-described in the literature.[6] On the basis of these parameters the distribution-pH profile of the four microspecies in aqueous solution can be characterized in detail. Nevertheless, some authors consider three protonation/deprotonation equilibria,[8] instead of two, while others report four pK_a values because of the existence of one carboxylic acid and three basic nitrogen sites in fluoroquinolones.[9]

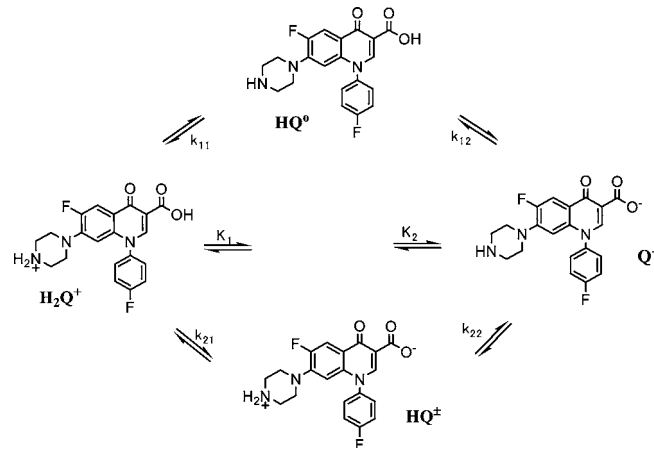

Figure 1. Protonation equilibrium of sarafloxacin. H_2Q^+, $HQ^{\pm}$, HQ^0, and Q^- represent the positive, zwitterionic, neutral, and negative microspecies, and k_{11}, k_{12}, k_{21}, and k_{22} and K_1 and K_2 represent micro- and macrodissociation constants, respectively.

According to Takacs-Novak et al.,[10] only the neutral species are lipophilic, and thus neglecting the solubility of the ions in the organic phase, log *P* may be calculated using the microspeciation information

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

Different experimental protocols to determine the apparent partition coefficient (log P_{app}) have been suggested in the literature.[11,12] In this work, the shake-flask method was used.

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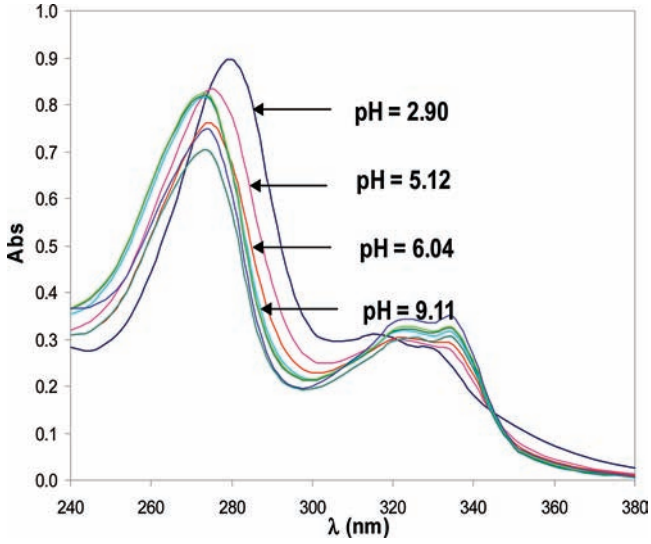

Figure 2. Spectra of sarafloxacin at different values of pH at 25 °C. The different pH values were obtained using the buffers described in the materials section.

Materials and Methods

Materials. The chemicals used in the measurements were sarafloxacin hydrochloride (LKT, 98 %), 1-octanol (Fluka, 99 %). All of the other reagents were at least analytical grade. Double-distilled water was used in the aqueous solutions.

Spectrophotometric Measurement of Protonation Equilibrium. Macroconstants and microconstants of protonation equilibrium were determined by spectrophotometry using a Shimadzu 1700 spectrophotometer, at room temperature. Solutions of fluoroquinolone (30 μM) were prepared with different values of pH and with a total ionic strength of 0.15 M by the addition of NaCl. The solutions with different pH values were obtained by mixing appropriate volumes of HCl (10^{-3} M) and NaOH (10^{-3} M). The pH measurements were undertaken using a pH meter (Hanna instruments HI 9321).

Determination of Apparent Partition Coefficient. The apparent partition coefficient was determined by the shake-flask[3,11] method in a 1-octanol/buffer system at pH 3.0, 5.0, 7.0, and 9.0 with a constant ionic strength of 0.15 M using NaCl. The buffer solutions used in this work were the following: acetate buffer pH 3.0, 0.1 M; acetate buffer pH 5.0, 0.2 M; phosphate buffer pH 7.0, 0.1 M; and borate buffer pH 9.0, 0.025 M. Solutions containing 10 $\mu g \cdot mL^{-1}$ of fluoroquinolone were prepared in the appropriate buffer, which was presaturated with 1-octanol. Equal volumes (5.0 mL) of the aqueous solution and 1-octanol (presaturated with the appropriate buffer) were mixed and placed in an orbital shaker, Stuart SSL1, at 200 rpm for 24 h. The orbital shaker is in an air bath at (25.0 ± 0.1) °C. Then the samples were centrifuged at 2500 rpm for 15 min, in an Eppendorf 5804 centrifuge, and the two phases were separated. The concentration of the solute was determined in the aqueous phase by spectrophotometry at the maximum absorption wavelength.

Results and Discussion

Evaluation of Protonation Equilibrium for Sarafloxacin. The spectrum of sarafloxacin in aqueous solutions at different values of pH has two completely separated and independent bands, as shown in Figure 2. The largest difference between the absorbance of the acid form and the conjugate basic form was found in the region around 280 nm. Thus, it is possible to determine the fraction of deprotonation of this group from the spectrum of absorption of fluoroquinolone as a pH function by eq 2.[6,13,14]

$$\alpha_{COO^-}(pH) = \frac{A_{(pH)} - A_{(COOH)}}{A_{(COO^-)} - A_{(COOH)}} \quad (2)$$

where $A_{(COO^-)}$ and $A_{(COOH)}$ are experimental values of absorbance when the carboxylic group is protonated and deprotonated, respectively, and $\alpha_{(COO^-)}$ is the fraction of deprotonation of the carboxylic group for a given value of pH, for which the absorbance is A (pH). The fraction of deprotonation ($\alpha_{(COO^-)}$) was determined at 284 nm. The macro- and microprotonation constants (pK_1, pK_2, and pk_{21}) were estimated from $\alpha_{(COO^-)}$ as a function of the pH data by an iterative nonlinear least-squares analysis[6,13,14] using the following equation:

$$\alpha_{COO^-}(pH) = \frac{k_{21}[H^+] + K_1K_2}{[H^+]^2 + K_1[H^+] + K_1K_2} \quad (3)$$

Other microprotonation constants (pk_{11}, pk_{12}, and pk_{22}) were then determined and are listed in Table 1. As mentioned above, the results published in the literature for the pK's of these compounds differ among different authors. However, the results of pK_1 and pK_2 obtained in this work are in good agreement with those published by Lutzhoft et al.[15] These values indicate that the carboxylic group in this compound is two orders of magnitude more acidic than other similar compounds, also presented in Table 1 for comparison. This behavior is due to the electron withdrawing effect of the 4-F

Table 1. Protonation Constants for Several Fluoroquinolones

fluoroquinolone		macroconstants		microconstants			
		pK_1	pK_2	pk_{11}	pk_{12}	pk_{21}	pk_{22}
sarafloxacin	this work	4.12	6.78	5.58	6.79	4.11	5.32
	ref 14	4.10	6.80				
	ref 17	5.62	8.18				
	ref 16	6.02	8.59				
difloxacina	ref 17	5.66	7.24				
ciprofloxacin	ref 13	6.09	8.62	8.74	6.09		
norfloxacin	ref 11	6.2	8.5	8.38	6.30		

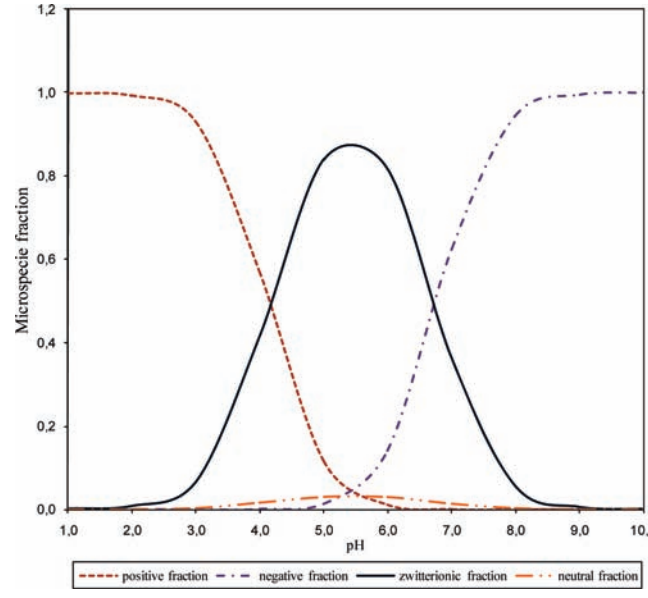

Figure 3. Distribution of four microspecies for sarafloxacin in the aqueous-phase system as a function of pH at 25.0 °C.

Table 2. log P_{app} Values of Sarafloxacin at Different pH Values

pH	log P_{app}	SD	*n*
3.0	−0.841	0.021	6
5.0	−0.652	0.020	6
7.0	−0.888	0.024	6
9.0	−1.261	0.001	6

phenyl group at the N_1 position, which ciprofloxacin and norfloxacin do not have since they present a cyclopropyl and an ethyl group in that position, respectively. For that reason the pK_1 and pK_2 measured by Renau et al.[16] and Jimenez-Lozano et al.[17] do not seem to be correct since they do not reflect the different structures of the fluoroquinolones. Nevertheless, it should be mentioned that the Jimenez-Lozano et al.[17] results for difloxacin are similar to those found by the same authors for sarafloxacin, showing internal consistency since these two fluoroquinolones are structurally very similar. Another interesting point of the microconstants pK_{21} and pK_{12} measured in this work is that they are practically identical to the macroconstants pK_1 and pK_2, indicating that the main route of protonation is conducted via neutral species.

On the basis of the protonation constants, the distribution-pH profile of the four microspecies in solution could be characterized in detail as shown in Figure 3. It is clear that the zwitterionic microform predominates over the neutral microspecies, and both reach a maximum at a pH around the isoelectric point (pI = 5.5). The ratio between the molar fraction of the neutral form and the zwitterionic form is equivalent to k_{11}/k_{21}. This value (for sarafloxacin is 3.63 %) is constant across the pH range and is characteristic of each fluoroquinolone.[14,15] Since only the neutral species are lipophilic,[8] a high ratio indicates that the compound has a large lipophilic character, determining its ability to reach the interior of the bacteria.[14,15]

Apparent Partition Coefficient (P_{app}). The apparent partition coefficient (P_{app}) was obtained from the following equation:[18]

$$P_{app} = \frac{A_i - A_f}{A_f} \cdot \frac{V_w}{V_o} \quad (4)$$

where A_i and A_f represent the absorbance of sarafloxacin in the aqueous phase before and after partitioning, respectively. V_w and V_o represent respectively the volume of the aqueous and organic phases used in the system octanol/water. The values of the apparent partition coefficient of sarafloxacin for different pH values studied and their standard deviation (SD) are shown in Table 2. The pH-partition profile, depicted in Figure 4, has a parabolic shape in an *n*-octanol/buffer system, which reflects the maximum lipophilicity at the sarafloxacin isoelectric point, pI = 5.5. For zwitterionic compounds, such as sarafloxacin, this parabolic shape[6,13,14] was expected, since the species with the smallest net charge is predominant at the isoelectric point. Below and above this pH, the apparent partition coefficients decrease, which indicates that fluoroquinolone polarity is higher and therefore the partition of sarafloxacin to the lipid phase is smaller.

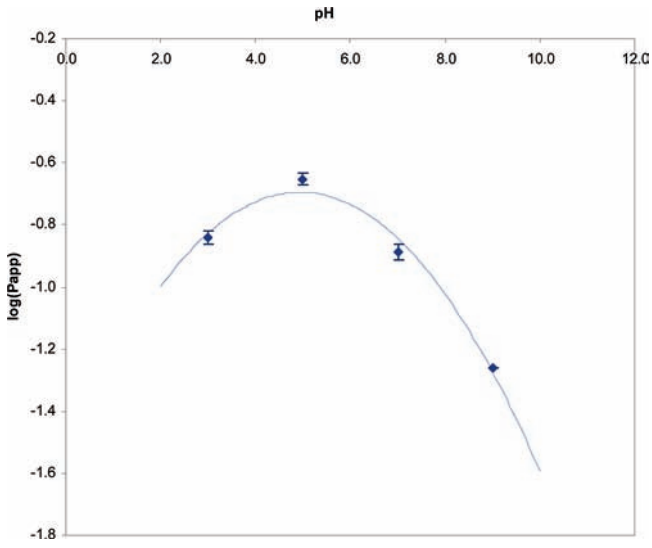

Figure 4. Apparent partition coefficient of sarafloxacin in an *n*-octanol/buffer system at 25.0 °C. The solid line represents the trend curve.

The true partition coefficient was defined on the basis of the apparent partition coefficient and the concentration of neutral species in solution, as described in eq 1. For the pH of the isoelectric point of fluoroquinolone, where the fraction of neutral species in aqueous solution is a maximum, the true partition coefficient (log P) can be determined.[3] For sarafloxacin the true partition coefficient in an *n*-octanol/buffer system (log P) is (0.864 ± 0.021). This value is similar to those published values for other well-studied fluoroquinolones, such as ciprofloxacin[11] (0.769 ± 0.089) and moxifloxacin[6] (0.832 ± 0.057).

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