ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
18 (1998) 859–863

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Study of acid–base equilibria of fleroxacin

G. Popović, Lj. Milovanović, V. Kapetanović *

Institute of Analytical and Inorganic Chemistry, Faculty of Pharmacy, University of Belgrade, P.O. Box 146, Vojvode Stepe 450, 11000 Belgrade, Yugoslavia

Received 15 May 1998; received in revised form 19 August 1998; accepted 30 August 1998

Abstract

The acid-base equilibria of fleroxacin were studied by means of potentiometry and spectrophotometry. It was established that fleroxacin undergoes a complex acid-base equilibrium due to its zwitterionic nature and two proton-binding sites of similar acidity. The stoichiometric equilibrium constants were determined at 25°C and constant ionic strength 0.1 M (NaCl). The acidity constants $pK_1 = 5.59 \pm 0.01$ and $pK_2 = 8.08 \pm 0.04$ were found by potentiometry, and $pK_1 = 5.61 \pm 0.03$ and $pK_2 = 8.11 \pm 0.06$ by spectrophotometry. The distribution diagram of the corresponding ionic species is given.

Keywords: Fleroxacin; Acidity constants; Spectrophotometry; Potentiometry

1. Introduction

Fleroxacin, belongs to the third generation of multiple fluorinated antibacterial quinolone derivatives widely used in the treatment of urinary infections. This generation members are of broader spectrum and greater activity compared to nalidixic and oxolinic acid [1–3]. The mechanism of their action was extensively studied [4]. These agents are proved to prevent bacterial DNA biosynthesis by inhibiting the bacterial enzyme DNA gyrase.

The behavior of fluoroquinolones are significantly influenced by their physicochemical properties, particularly by their ionization degree expressed by the pK_a value and partition coefficient [5,6]. These data are important for a thorough understanding of absorption transport and receptor binding of these drugs at the molecular level.

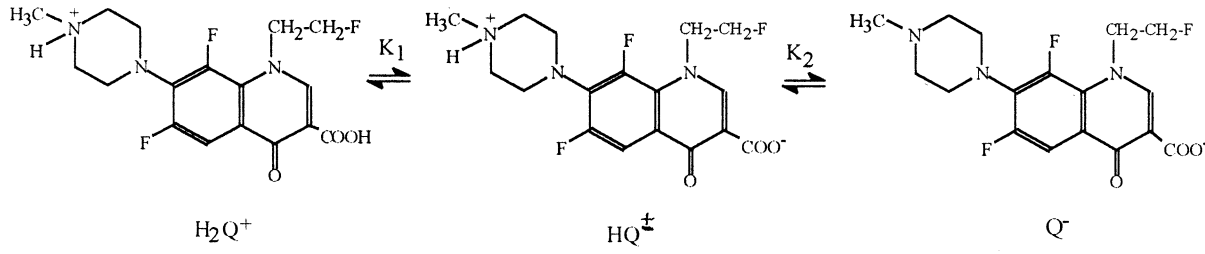
Fleroxacin is 6,8-difluoro-1-(2-fluoro ethyl)-1,4-dihydro-7-(methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid.

Acid–base equilibrium of several fluoroquinolones has been reported in literature recently [6,7]. Fleroxacin as the other fluoroquinolones have two potentially ionizable functional groups, namely the carboxylate and piperazine amino groups.

In the present work the acid–base properties of fleroxacin was studied by potentiometry and UV spectrophotometry. Since these molecules contain two proton-binding sites of similar basicity, the acid–base properties are depicted by equilibrium constants, K_1 and K_2, respectively.

* Corresponding author.

0731-7085/98/$ - see front matter
PII: S0731-7085(98)00281-7



Scheme 1. Protonation Scheme of fleroxacin.

2. Experimental

2.1. Apparatus and reagents

The following apparatus was used: a PHM-62 pH meter (Radiometer, Copenhagen, Denmark) with a glass-calomel electrode assembly; titrator TTT-60 with an ABU-12 autoburette (Radiometer); and Spectrophotometer Beckman DU 650.

All investigations were carried out with fleroxacin produced by Hoffmann La Roche (Basel, Switzerland). Other reagents, HCl, NaOH and NaCl were of analytical reagent grade from Merck. Double distilled water was used throughout. Standardization of HCl and NaOH was performed potentiometrically. The ionic strength was kept constant (0.1 M) by addition of NaCl, and all measurements were performed at 25°C. The conversion of measured pH values into pc_H for $I = 0.1$ M was done by using the following relation [8]:

$$pc_H = -\log [H_3O^+] = pH - 0.04.$$

2.2. Determination of acidity constants

2.2.1. Potentiometric determination of acidity constants

The acidity constants were determined from the data obtained by potentiometric titration. Aliquot (25 ml) containing 2.5×10^{-3} M fleroxacin and 3.882×10^{-3} M HCl were titrated by 0.1454 M NaOH. The constant ionic strength of 0.1 M was kept by NaCl.

2.2.2. Spectrophotometric determination of acidity constants

Two aliquots of 5×10^{-5} M fleroxacin solutions were prepared in either 1×10^{-2} M HCl or 1×10^{-2} M NaOH with a total ionic strength of 0.1 M (NaCl). By mixing the acidic and basic solutions, the solutions of different pH were obtained and their spectra were recorded in the wavelength range of 220 to 400 nm.

3. Results and discussion

The neutral nonionic form of fleroxacin is rearranged spontaneously to the zwitter ion due to the protolysis of the carboxyl group and the proton acceptance of the piperazine-amino group. In the pH range 2–12 fleroxacin as a zwitter ion undergoes a complex acid–base equilibrium as shown in Scheme 1, where $HQ^{\pm}$, H_2Q^+ and Q^- represent a zwitter, a cation and an anion species, respectively. The corresponding equilibrium constants are as follows:

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

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

Potentiometric determination of equilibrium constants was performed by application of the formation function method [9]. The method is based on the determination of $\bar{n}$, i.e. the average number of protons bound to the free base. In the case examined it is given by:

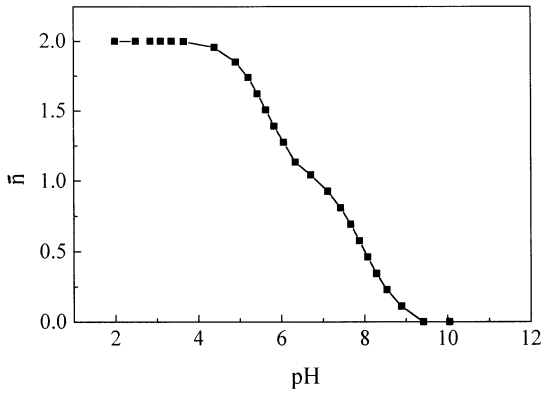


Fig. 1. Formation function dependence on pH.

$$\bar{n} = \frac{2[H_2Q^+] + [HQ^\pm]}{[H_2Q^+] + [HQ^\pm] + [Q^-]} \quad (3)$$

By combining Eqs. (1) and (2) and Eq. (3), the linear dependence was obtained:

$$\frac{\bar{n}-1}{\bar{n}}[H^+] = \frac{1}{K_1}\frac{2-\bar{n}}{\bar{n}}[H^+]^2 - K_2 \quad (4)$$

on the basis of which K_1 and K_2 can be determined from the slope and intercept, respectively.

The determination of the $\bar{n}$ from experimental data was calculated according to the equation:

$$\bar{n} = \frac{c_{HQ} + c_{HCl} - c_{NaOH} - [H^+] + [OH^-]}{c_{HQ}} \quad (5)$$

where c_{HQ}, c_{HCl} and c_{NaOH}, correspond to the stoichiometric concentrations of fleroxacin, hydrochloric acid and sodium-hydroxide solution respectively; $[H^+]$ and $[OH^-]$ were obtained from pH measurements.

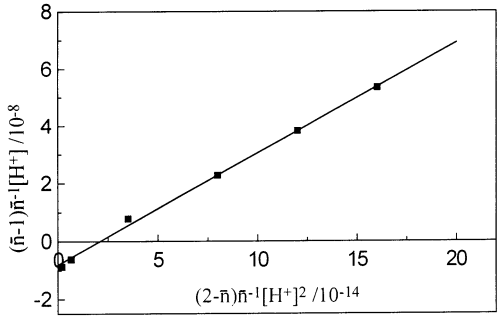


Fig. 2. Graphical presentation of Eq. (4) for potentiometric evaluation of ionization constants K_1 and K_2.

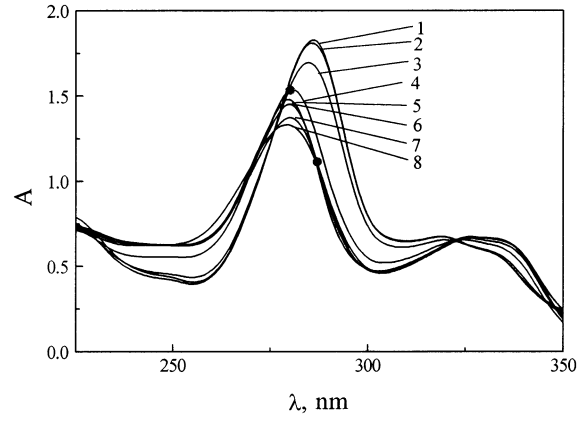


Fig. 3. The absorption spectra of fleroxacin obtained in: 1, 0.01 M HCl (H_2Q^+); 2, pH 3.50; 3, pH 4.95; 4, pH 5.98; 5, pH 6.85; 6, pH 8.25; 7, pH 9.00; 8, 0.01 M NaOH (Q^-).

The dependence of formation function, $\bar{n}$ versus pH is shown in Fig. 1.

Linear dependence of $(\bar{n}-1)\bar{n}^{-1}[H^+]$ versus $(2-\bar{n})\bar{n}^{-1}[H^+]^2$ gives $K_1 = 2.58 \times 10^{-6}$ ($pK_1 = 5.59$), and $K_2 = 8.23 \times 10^{-9}$ ($pK_2 = 8.08$) (Fig. 2).

The absorption spectra of fleroxacin obtained in pH range from 2 to 12 are shown in Fig. 3.

The spectra of H_2Q^+ species (pH 2) and spectra of fleroxacin obtained at higher pH values, up to pH 5.98, pass through the isosbestic point at 279 nm, since the spectra of Q^- species (pH 12) and the spectra of fleroxacin obtained at lower pH values, up to 6.85, show the second isosbestic point at 286 nm. Since the classic spectrophotometric approach [10] in investigation of the acidity constants demands the spectra of all pure species to be known, the application of this method was not possible due to the overlapping acid–base equilibrium of fleroxacin. In this case it was possible to estimate the spectra of two pure species, H_2Q^+ and Q^-, at pH < 3.6 and pH > 10, respectively. Because of that, for determination of K_1 and K_2 of fleroxacin the basic spectrophotometric equations:

$$pK_1 = pc_H + \log\frac{A - A_{HQ^\pm}}{A_{H_2Q^+} - A} \quad (6)$$

$$pK_2 = pc_H + \log\frac{A - A_{Q^-}}{A_{HQ^\pm} - A} \quad (7)$$

were transformed into the following linear dependences:

$$A = A_{HQ^{\pm}} + \frac{1}{K_1}(A_{H_2Q^+} - A)\,[H^+] \quad (8)$$

and $$A = A_{HQ^{\pm}} + K_2 \frac{A_{Q^-} - A}{[H^+]} \quad (9)$$

According to Eqs. (8) and (9) the estimation of K_1 and K_2 is possible on the basis of A—pH data, knowing the absorbance of only one pure species, H_2Q^+ and Q^- , respectively. The Eqs. (8) and (9) represent linear dependence of $A = f\{(A_{H_2Q^+} - A)[H^+]\}$ and $A = f\{(A_{Q^-} - A)/[H^+]\}$ on the basis of which K_1 and K_2 were calculated by linear regression from the slopes of the corresponding graphs , Fig. 4. Determination of K_1 was performed at 290 nm in pH range from Eq. (2) to 5.93, and K_2 at 280 and 290 nm in pH range from 7.90 to 12.

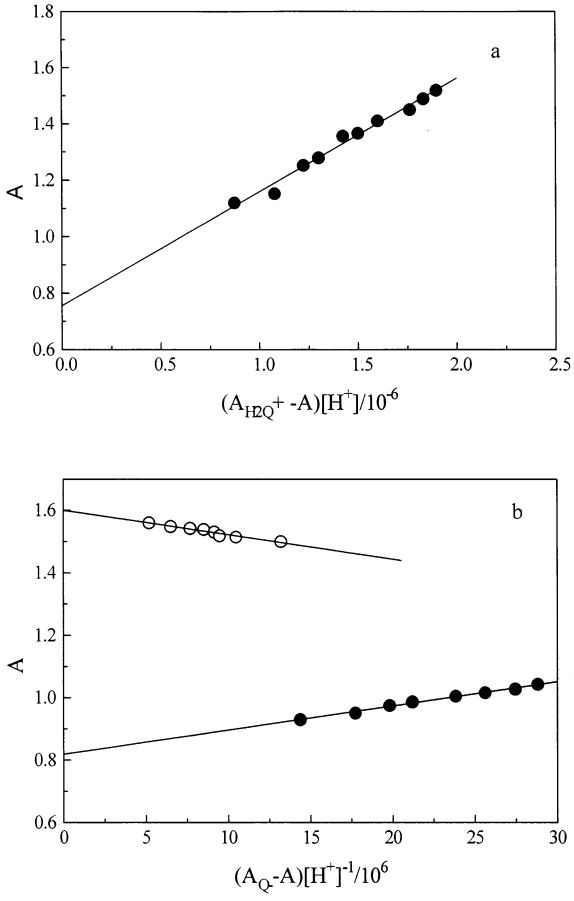


Fig. 4. Graphical presentation of Eqs. (8) and (9) for spectrophotometric determination of ionisation constants: (a) K_1 (290 nm); (b) K_2 (○ ○ ○, 280 nm; ● ● ●, – 290 nm).

Table 1
Stoichiometric ionization constants of fleroxacin[a]

Constant	Method applied	
	Potentiometry	Spectrophotometry
pK_1	5.59 ± 0.01	5.61 ± 0.03
pK_2	8.08 ± 0.04	8.11 ± 0.06
pI[b]	6.84	6.86

[a] $I = 0.1$ M (NaCl), $t = 25$°C.
[b] Isoelectric point.

The equilibrium constants obtained by the potentiometric and spectrophotometric methods are summarized in Table 1. A good agreement of K_1 and K_2 is obtained with both methods. The spectrophotometric method is a less precise method for determination of K_2 compared to pH-metric method as seen from Table 1. This can be ascribed to the small change of the quinolone spectrum caused by the piperazine moiety protonation. An extensive literature review [6,7] related to the numerical values of the ionization constants of fluoroquinolones show that the values for pK_1 and pK_2. are approximately 5.3–6.5 and 7.6–9.3, respectively. According to Ross and Riley [6] in the case of single fluorinated quinolones (at C-6 position) the pK_1 and pK_2 of both ionization centres are considered in detail , related to the influence of the fluorine and 4′-*N* methyl supstituents. By introducing the second fluorine atom into the molecule of quinolone (at C-8 position), i.e. in the case of lomefloxacin, 8-fluoro-norfloxacin and 8-fluoro pefloxacin, the pK_1 varies from 5.33 – 5.55 [7]. The value of pK_1 obtained for fleroxacin is in a good accordance to those values. At the same time, the pK_2 for lomefloxacin and 8-fluoronorfloxacin are 8.78 and 9.33, respectively. The pK_2 of 8-fluoro pefloxacin which contains 4′-*N* methyl supstituent is 8.13. This value can be compared with the pK_2 of fleroxacin, due to their very similar structure (the difference is in fluorine atom of side ethyl chain in the molecule of fleroxacin). This findings show

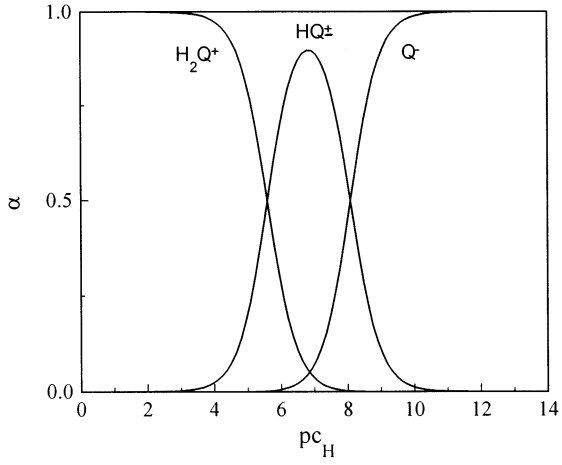


Fig. 5. The distribution diagram of fleroxacin.

probably the minor influence of the fluorine side chaine atom to both ionizable centres.

On the basis of the determined constants, the relative concentration of the equilibrium species can be calculated using the following equations:

$$\alpha_{H_2Q^+} = \frac{[H^+]^2}{[H^+]^2 + K_1[H^+] + K_1K_2} \quad (10)$$

$$\alpha_{HQ^\pm} = \frac{K_1[H^+]}{[H^+]^2 + K_1[H^+] + K_1K_2} \quad (11)$$

$$\alpha_{Q^-} = \frac{K_1K_2}{[H^+]^2 + K_1[H^+] + K_1K_2} \quad (12)$$

The corresponding distribution diagram of fleroxacin is presented in Fig. 5.

The literature data show that antibacterial activity of fluoroquinolones is pH dependent [11]. For this reason, the fluoroquinolones distribution can be utilazed for better understanding and interpretation of the protein binding of these drugs. The poor binding of the zwitterionic form has been recently reported [12]. Besides, a progressive decrease of the activity of some quinolones at low pH can be atributed to the poor penetrating of the cationic through the cell membrane [13,14].

References

[1] J.R. Prous, N.E. Mealy, Drugs Today 19 (1983) 339–341.
[2] R. Albrecht, Prog. Drug Res. 21 (1977) 9–104.
[3] H. Koga, Gekkan Yakuji 25 (1983) 675–680.
[4] J.M. Domagala, L.D. Hann, C.L. Heifetz, M.P. Hutt, T.F. Mich, J.P. Sanchez, M. Solomon, J. Med. Chem. 29 (1986) 394–404.
[5] G.E. Stein, Am. J. Med. 82 (S–6B) (1987) 18–21.
[6] D.L. Ross, C.M. Riley, J. Pharm. Biomed. Anal. 12 (1994) 1325–1331.
[7] K. Takacs-Novak, B. Noszal, I. Hermecz, G. Kereszturi, B. Podanyi, G. Szasz, J. Of. Pharm. Sci. 79 (1990) 1023–1028.
[8] L.B. Pfendt, D.M. Sladić, T.J. Janjić, G.V. Popović, Analyst 115 (1990) 383–387.
[9] F.J.C. Rossotti, H. Rossotti, The Determination of Stability Constants, McGraw-Hill, New York, 1961, p. 110.
[10] A. Albert, E.P. Serjent, The Determination of Ionization Constants A Laboratory Manual, Chapman and Hall, London, 1984.
[11] M. Neumann, A. Esanu, Drugs Exp. Clin. Res. 19 (1988) 385–391.
[12] T. Izumi, T. Nagayama, T. Kitagawa, Chem. Pharm. Bull. 37 (1989) 746–752.
[13] B. Holmes, R.N. Brogden, D.M. Richards, Drugs 30 (1985) 482–513.
[14] J.B. Cornett, R.B. Wagner, A.R. Dobson, M.P. Wentland, D.M. Bailey, Antimicrob. Agents Chemother. 27 (1985) 4–10.