Pergamon

Dissociation and complexation of fluoroquinolone analogues

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Abstract: The dissociation and the complexation behaviours of four fluoroquinolone antibiotics have been studied. The acid dissociation constants of ciprofloxacin, enoxacin, norfloxacin and ofloxacin were determined by conventional potentiometric and conductometric techniques. Increasing the Hammett substituent constant, the pK_a values were decreased. The absorption of fluoroquinolones in the intestinal tract are probably transported by pH-dependent mechanisms. Formation constants of the iron(III) complexes (1:1) of the fluoroquinolone analogues were determined by spectrophotometry. The optimum pH for complexation was 3.80.

Introduction

Quinolone antibiotics such as ciprofloxacin [1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid], enoxacin [1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid], norfloxacin [1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid] and ofloxacin [9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid] are all synthetic, orally active, broad-spectrum agents effective against resistant mutants of bacteria [1-3]. The parent compound of these drugs is 6-fluoro-4-oxoquinoline-3-carboxylic acid. These analogues known as the trivial name fluoroquinolones are all fluorinated in position 6 and bear a piperazinyl moiety in position 7.

Information about ionization of antibiotics can be used for evaluation of physicochemical properties and biological activities. The equilibrium constant can also be used for the calculation of the composition of a system in equilibrium and completeness of the chemical reaction could be estimated. It is also possible to evaluate the salt effect, the change of the equilibrium position as function of temperature and, if necessary, to estimate the influence of various side-reactions on the equilibrium during certain stages of the analytical procedure [4, 5]. Studies on the dissociation and the complexation are also very important to understand the transportation of drugs. The present work was performed to investigate the behaviour of dissociation and complexation of fluoroquinolone antibiotics with iron(III).

Experimental

Reagents

Ciprofloxacin hydrochloride monohydrate (Bayer Korea, purity 98.3%), enoxacin (Dong-A, purity 99.90%), norfloxacin (Sam-A, purity 100.0%), ofloxacin (Jeil, purity 100.1%), and nalidixic acid (Sudo; Unibios, purity 100.2%) were kindly provided by their manufacturers. Water used in the experiments was distilled-deionized and then purified using a E-pure water purification system (Barnstead). The specific conductivity of this water was 1.8×10^{-7} ohm^{-1} cm^{-1}. All other chemicals were of reagent grade and purchased from Merck or Sigma.

Instruments

Absorption spectra were recorded on a Hewlett–Packard HP8452 diode array spectrophotometer linked with a HP-IB computer

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interface. IR spectra were obtained by a Perkin–Elmer 1310 IR-spectrophotometer. The fluorescence of solutions in a 10 mm quartz cell was measured at the corresponding maximum excitation and emission wavelengths bandpath settings (10 nm) by a Hitachi model F-2000 scanning spectrofluorometer with 150 W xenon lamp. An Orion SA-520 pH meter equipped with combination electrode was used for pH or potential (mV) measurements. Conductance measurements were made by using a Yellow Springs YSI 3417 conductance meter. All glassware was thoroughly cleaned, rinsed several times with water and/or methanol.

Procedures

Determinations of pK_a values were carried out according to conventional potentiometric or conductometric techniques [6]. On the day of the experiment, 1.0×10^{-3} M–7.5×10^{-5} M aqueous fluoroquinolone analogue solutions according to their solubility were titrated with a 3×10^{-3} M–7.5×10^{-5} M sodium hydroxide standard solution.

The ionic strength of the samples were kept constant and equal to 1.0×10^{-1} M with sodium nitrate. Otherwise a 300.0 mg sample was dissolved with 30.0 ml acetic acid glacial, and then was titrated with 0.1 N perchloric acid. The volume and pH or conductance was recorded at each point. The analysis was performed in triplicate at least.

A volume (5 ml) of 3.0×10^{-4} M fluoroquinolone analogue standard solution was added to 5 ml of 3.0×10^{-4} M ferric nitrate nonahydrate solution, then the ionic strength was kept constant (1.0×10^{-1} M) with sodium nitrate and the pH was adjusted with nitric acid. Formulae and stability constants of soluble reddish yellow complexes formed between ferric nitrate and the ligand fluoroquinolone analogues were determined spectrometrically by continuous variations, molar ratio method, Bjerrum's method and/or Scatchard plot [7–11].

Results and Discussion

Acid dissociation constant

Fluoroquinolone analogues have two relevant ionizable functional groups, which means that their acid–base chemistry involves two protons. Both nalidixic acid and enoxacin have naphthyridine rings, but other analogues have

quinoline rings. In contrast with nalidixic acid, the fluoroquinolone analogues have a basic piperazinyl group in the 7-position and carboxylic acid group in the 3-position.

The protolytic equilibria of fluoroquinolone analogues are expressed in Scheme 1. Fluoroquinolone can exist in four possible forms such as an acidic cation (H_2Q^+), a neutral unionized species (HQ°), an intermediate zwitter ion ($HQ^\pm$) and a basic anion (Q^-), depending on the given pH. At a low pH, both the 7-piperazinyl group and 3-carboxyl group are protonated. At a high pH, neither is protonated [1].

The carboxyl group is normally a stronger acid than the ammonium group. Therefore, the neutral nonionic form is rearranged spontaneously to the zwitter ion. The total ampholyte concentration, [HQ] is almost the same as [$HQ^\pm$] on the assumption that [HQ] = [HQ°] + [$HQ^\pm$], where [HQ°] is approximately negligible under the experimental conditions. Accordingly, the macroscopic dissociation constants (K_{a_1}, K_{a_2}) can be defined by the following equations.

$$K_{a_1} = [HQ][H^+]/[H_2Q^+],$$

$$K_{a_2} = [Q^-][H^+]/[HQ].$$

The first K_a value applies to the 3-carboxyl proton, and the last is for the 7-piperazinyl proton.

The acid dissociation constants of four fluoroquinolones were determined potentiometrically and conductometrically at an ionic strength of 0.1 at 25°C. The apparent pK_a values determined are listed in Table 1. Low dissociation constants may contribute to the insolubility of these antibiotics.

Published data on the dissociation behaviours are limited. Two decades ago, pK_a values of nalidixic acid were determined spectrophotometrically by Staroscik *et al.* [12]. According to their report, the pK_{a_1} value of 0.94 corresponds to the dissociation of protonated heterocyclic nitrogen (8-position) of nalidixic acid, while pK_{a_2} of 6.02 corresponds to the dissociation of the carboxylic acid group. Our results are in good agreement with the data of Staroscik *et al.* However pK_a values of norfloxacin are different from the given values (6.34 and 8.75) in the previous reports by Mauzel [13] and Superlock [14]. Probably the difference was due to methodology because
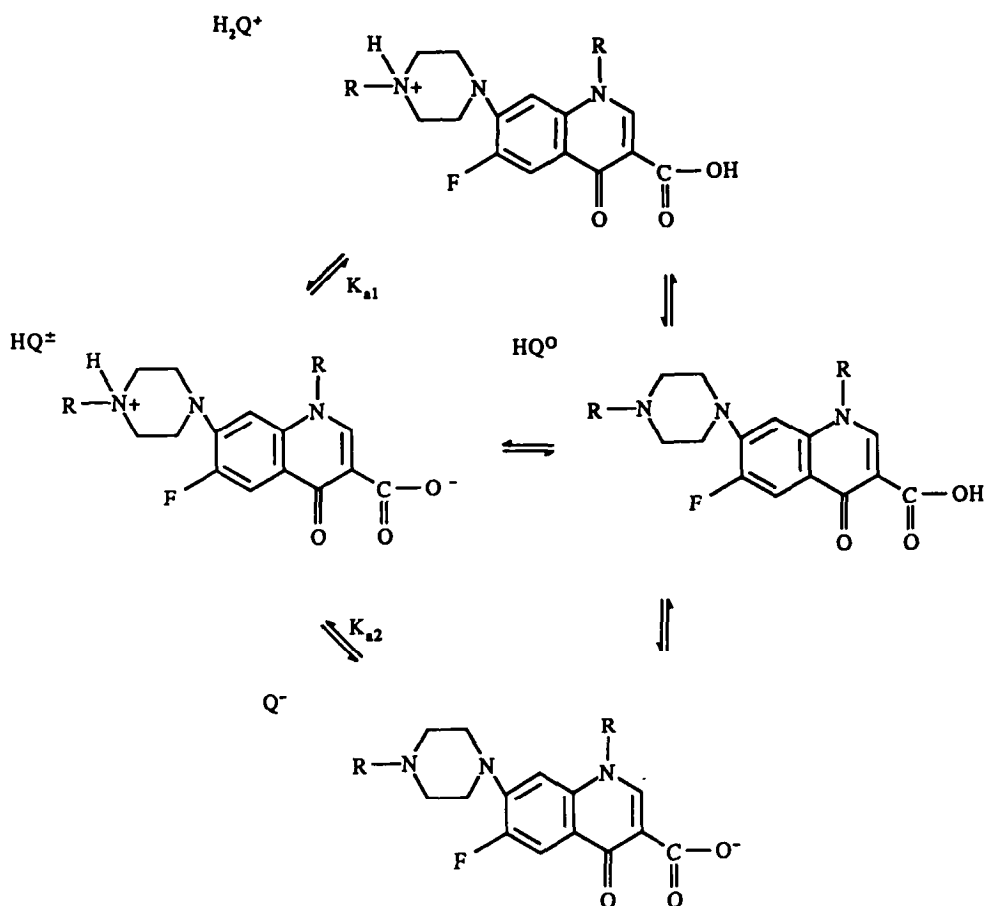
Scheme 1
The dissociation of fluoro-oxo-quinoline carboxylic acid analogues.

Table 1
pK_a values of fluoroquinolone analogues

Compounds	$pK_{a_1} \pm SD$	$pK_{a_2} \pm SD$
4-Oxo-quinoline [6]	2.23	11.28
Ciprofloxacin	6.14 ± 0.03	7.41 ± 0.02
Norfloxacin	0.74 ± 0.01	8.26 ± 0.02
Ofloxacin	0.90 ± 0.02	7.88 ± 0.02
Nalidixic acid	0.95 ± 0.01	6.36 ± 0.01
Enoxacin	0.71 ± 0.01	6.71 ± 0.03

our values (pK_1) were calculated from the data of non-aqueous potentiometry (Fig. 1), but their data from aqueous solution.

Effect of N-1 substituents on the dissociation

The inductive effect of substituents in the N-1 position of the fluoroquinolone ring is defined as following Hammett correlation [15, 16]:
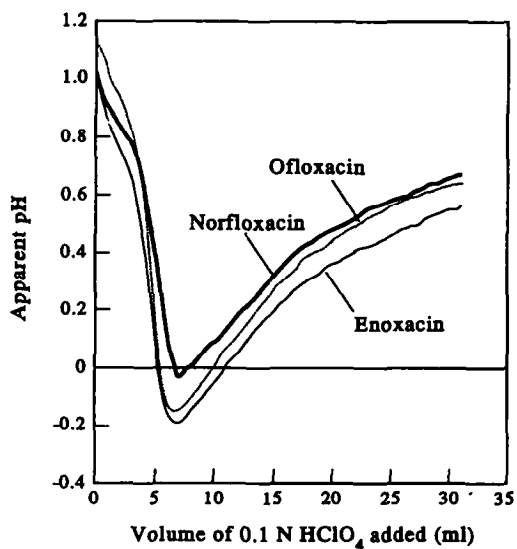
$$\log (K_a^x/K_a^q) = \sigma \rho,$$

Figure 1
Experimental potentiometric non-aqueous titration curves for 3.1×10^{-2} M enoxacin, 3.1×10^{-2} M norfloxacin and 2.8×10^{-2} M ofloxacin treated with 0.1 M HClO$_4$.

thus

$$pK_a^x = pK_a^q - \sigma \, \rho,$$

where K_a^x = acid dissociation constant of fluoroquinolone analogues, K_a^q = acid dissociation constant of 4-oxo-quinoline, σ = Hammett substituent constant and ρ = reaction constant. The σ vs pK_a profile for ciprofloxacin, norfloxacin, and ofloxacin is given in Fig. 2 when it was presumed that ρ was unity. Increaisng the substituent constant σ, the pK_a values were decreased. This indicates that the dissociation process of fluoroquinolone is greatly dependent upon the N-1 substituents.

Fractional distribution

Figure 3 shows the curve for the fractional composition for fluoroquinolone analogues which have two pK_a values. At pH < pK_{a_1}, both the piperazinyl group and the 3-carboxyl group are protonated, while at pH > pK_{a_2}, neither is protonated. Under the optimum

Figure 2
Correlation between Hammett substituent constant and pK_a.
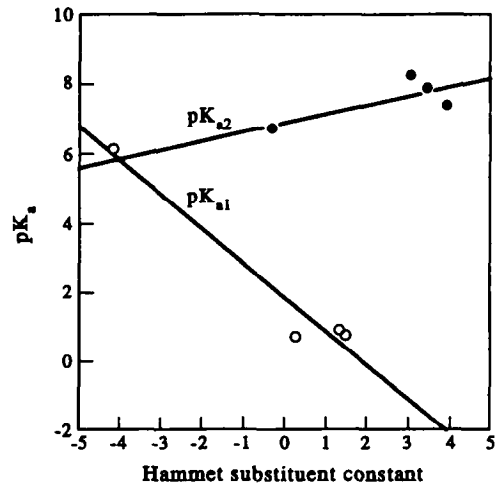
condition of the complexation with iron(III) at pH 3.80, the acidic cation of ciprofloxacin is the predominant form whereas for enoxacin, norfloxacin and ofloxacin, intermediate zwitter ions are the major forms.
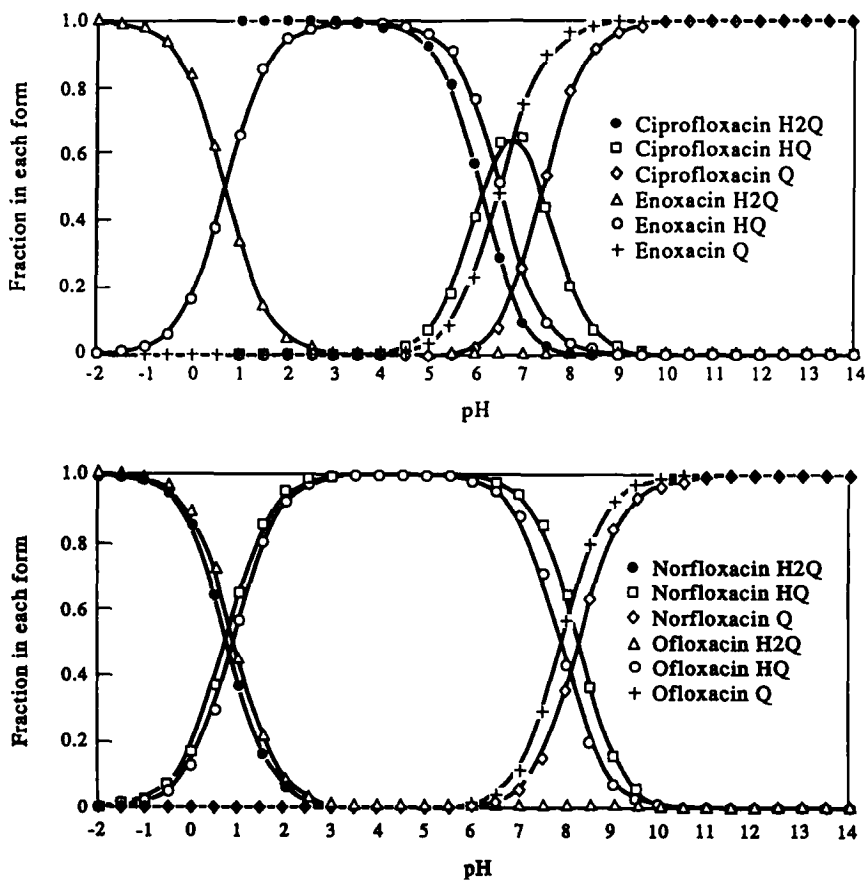

Figure 3
(a) Fractional distribution diagrams for ciprofloxacin and enoxacin. (b) Fractional distribution diagrams for norfloxacin and ofloxacin.

Fractional compositions of drugs are necessary to understand stability and transportation models in the gastrointestinal and biological membrane [17]. This transportation depends on the lipid solubility and degree of ionization of the antibiotics. These terms are of course a function of the pH of the absorptive medium and pK_a values of the antibiotics [18]. For acids with pK_a values between 2.5 and 7.5, pH-dependent absorption is expected whereas weak acids with pK_a values greater than 7.5 and bases with pK_a values less than 5 have pH-independent absorption [19]. According to the present observation the absorption of fluoro-quinolone analogues in the intestinal tract may be transported by pH-dependent mechanisms.

Absorption spectra

Fluoroquinolone reacts with iron(III) by forming water soluble reddish-yellow complexes. The UV–vis spectra of iron(III) complexes at pH 3.80 are shown in Fig. 4. The maximum wavelengths of those excitation and fluorescence spectra are listed in Table 2. Absorption maxima are shifted to longer wavelengths by complexation.

IR spectra of fluoroquinolone analogues and those iron(III) complexes after purification
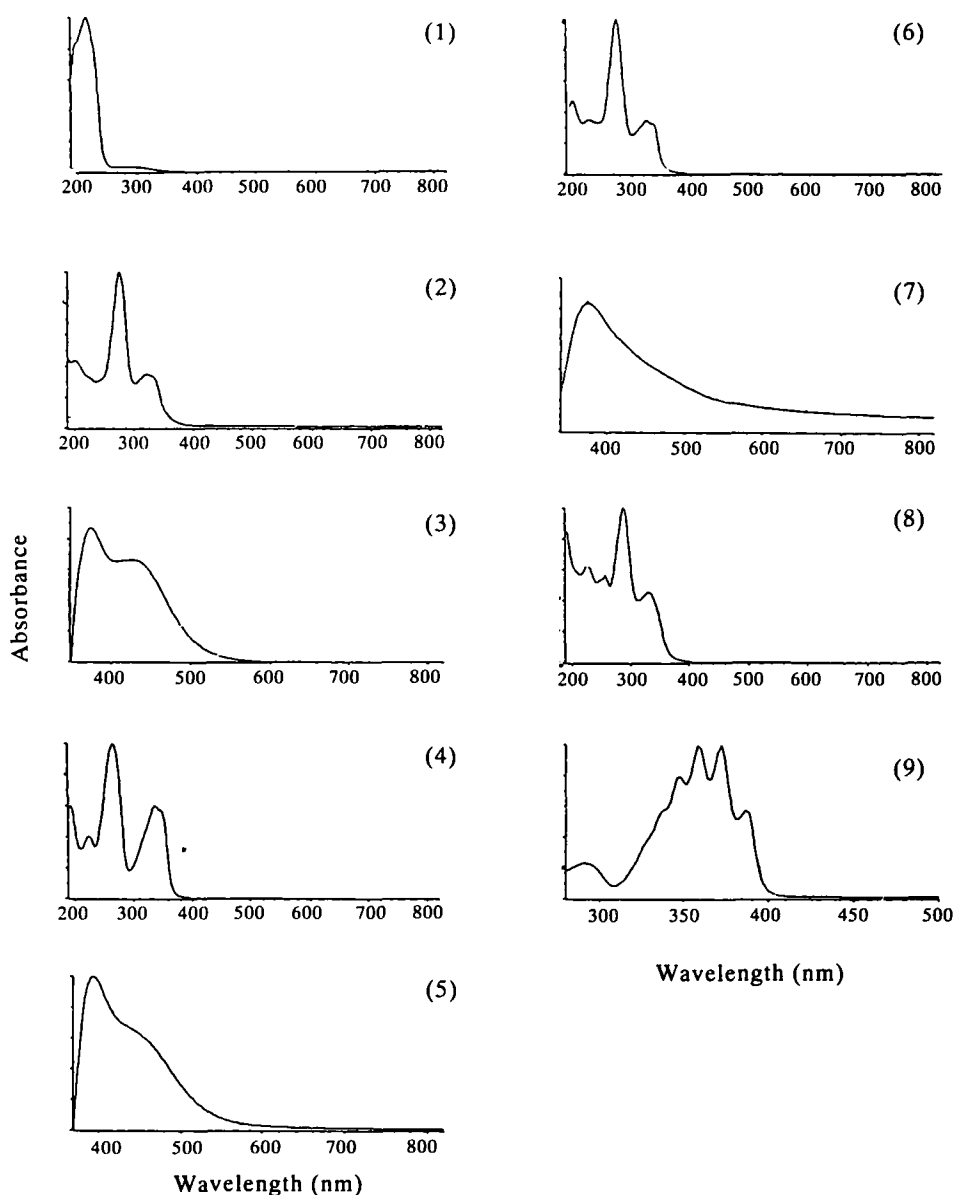

Figure 4
UV–vis spectra of (1) ferric nitrate, (2) ciprofloxacin, (3) iron(III) complex of ciprofloxacin, (4) enoxacin, (5) iron(III) complex of enoxacin, (6) norfloxacin, (7) iron(III) complex of norfloxacin, (8) ofloxacin and (9) iron complex of ofloxacin.

Table 2
UV–vis and fluorescent properties of fluoroquinolones and their iron(III) complexes

| Compounds	UV–vis spectra in water λ_{max} (nm)	Fluorescence spectra			
		in sulphuric acid		in water	
		λ_{ex} (nm)	λ_{em} (nm)	λ_{ex} (nm)	λ_{em} (nm)
Ciprofloxacin	276	279	447	278	442
Iron(III) complex of ciprofloxacin	374	320	450	278	446
Enoxacin	266	279	338	273	397
Iron(III) complex of enoxacin	394	312	552	341	404
Norfloxacin	278	281	445	279	444
Iron(III) complex of norfloxacin	374	318	446	279	443
Ofloxacin	288	294	494	292	495
Iron(III) complex of ofloxacin	372	342	501	292	495
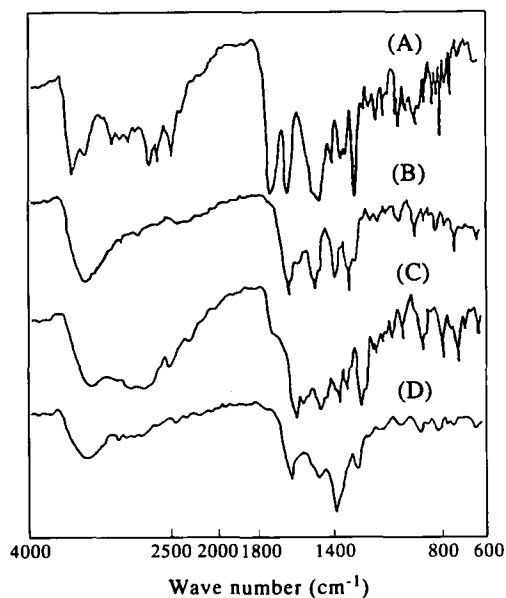
Figure 5
IR spectra of (A) ciprofloxacin, (B) iron(III) complex of ciprofloxacin, (C) norfloxacin and (D) iron(III) complex of norfloxacin.

were recorded by KBr disk method. Absorption bands of complexes were shifted to short wavenumber (Fig. 5). These changes in the absorption bands suggested that probable metal ligating sites are C-3 carboxyl -OH group and C-4 oxo group.

Composition of complexes

The stoichiometric compositions of fluoroquinolone analogues in the iron(III) complexes have been established by the Job's method of continuous variations, molar ratio method. The curves obtained (Fig. 6) had maxima at a mole fraction of 0.5 which indicated the formation of 1:1 (metal:ligand) complex in every case. The results obtained by the molar ratio method are shown in Fig. 7; it is also
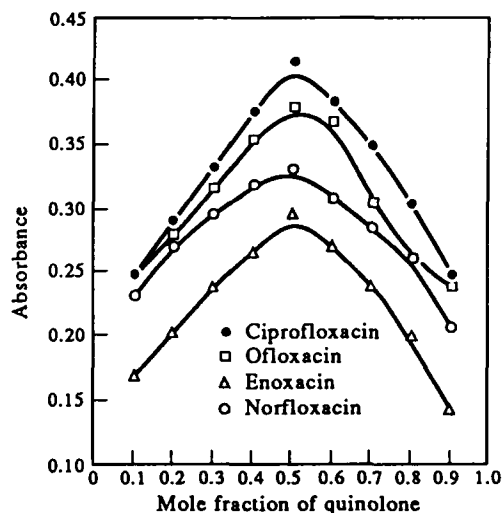

Figure 6
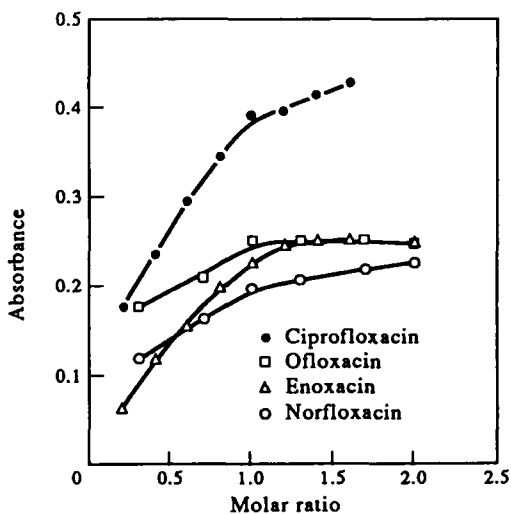Continuous-variation plot for the determination of the formula.


Figure 7
Molar ratio plot for the determination of the formula.

confirmed that fluoroquinolone analogues form 1:1 complexes with iron(III). Our results are in good agreement with the data of Jelikic-Stankov et al. [20]. However according to the previous literature by Issopoulos [21] the iron(III) complex of norfloxacin has 1:2 composition, in which ammonium iron(III) sulphate solution was used with a pH value under 3.0 and the ionic strength, ca 0.3 M maintained with 0.1 M ammonium sulphate but no data were presented.

Formation constant of complexes

Siddall and Vosburgh [22] made a series of absorbance measurements on 1×10^{-4} M iron solutions in the acidity range of $[H^+] = 0.001-0.015$, and it appeared that in this range the only significant iron(III) species were $FeOH^{2+}$ and Fe^{3+}. At optimum pH 3.80, the acidic cation of ciprofloxacin is predominant form whereas intermediate zwitter ions of enoxacin, norfloxacin and ofloxacin are the major forms. From these data, the formation constants of complexes (1:1) could be defined as follows if the formation of only mononuclear species is assumed.

$$Fe^{3+} + HQ^{\pm} = FeQ^{2+} + H^+,$$
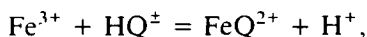
$$K_f = [FeQ^{2+}]/[Fe^{3+}][HQ^{\pm}].$$

The formation constant (K_f) of complexes have been determined by the application of Bjerrum's method and the Scatchard plot [10, 11]. The results obtained are shown in Fig. 8 and are listed in Table 3. Higher formation constants of iron(III) complexes of fluoroquinolone analogues indicate its stability. Our data on the formation constant of norfloxacin with iron(III) is lower than the previous report by Issopoulos [21].

Effect of pH

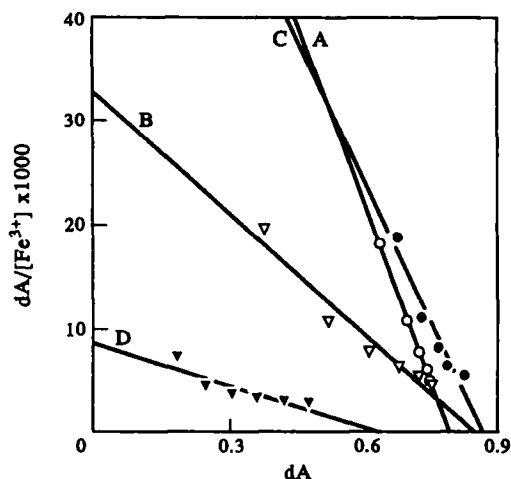The effect of pH on the absorbances of the complexes was examined, as shown in Fig. 9.

Figure 8
Scatchard plot of iron(III) complexes of fluoro-oxoquinoline. (A) Ciprofloxacin, (B) enoxacin, (C) ofloxacin, (D) norfloxacin.
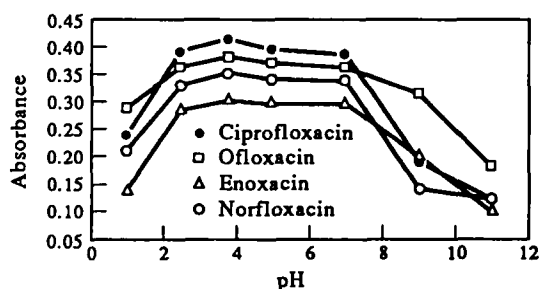

Figure 9
Effect of pH on complex formation: absorbance of complexes as a function of pH.

Constant absorbances were obtained over the pH range from 2.50 to 7.00 and the optimum pH of the complexation was 3.80. The complexation would be inhibited at stronger acidic pH range below 2.50 because the dissociations of fluoroquinolone analogues are suppressed. In the case of alkaline media the hydrolysis [23] of the iron(III) showed interference with the formation of complexes.

Table 3
Formation constants of complexes calculated according to Bjerrum's method and Scatchard plot

Compound	$C_Q = C_{Fe}$ (M)	Bjerrum's method				Scatchard plot $K_f \pm$ SD
		A_{calp}	A	[FeQ]	$K_1 \pm$ SD	
Ciprofloxacin	7.5×10^{-5}	0.0448	0.4147	6.99×10^{-5}	$2.71 \,(\pm 0.05) \times 10^6$	$3.49 \,(\pm 0.08) \times 10^5$
Enoxacin	7.5×10^{-5}	0.3062	0.2953	7.23×10^{-5}	$1.01 \,(\pm 0.03) \times 10^7$	$3.91 \,(\pm 0.02) \times 10^4$
Norfloxacin	7.5×10^{-5}	0.3487	0.3305	7.11×10^{-5}	$4.64 \,(\pm 0.02) \times 10^6$	$1.41 \,(\pm 0.06) \times 10^4$
Ofloxacin	7.5×10^{-5}	0.3931	0.3787	7.23×10^{-5}	$9.57 \,(\pm 0.08) \times 10^6$	$3.49 \,(\pm 0.04) \times 10^5$

Stability of absorbance and effect of foreign ions

The iron(III) complexes of fluoroquinolone analogues were formed immediately after addition of the reagents. The absorbance of the complexes remained stable without any change for 1 h at ambient temperature under the proposed method. Neither nickel(II), cobalt(II), magnesium(II) or zinc(II) interfered with the complexation when 10 ppm were added. However complexation was interfered by copper(II).

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