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Chemical and microbiological investigations of metal ion interaction with norfloxacin

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Summary

This report describes a spectrophotometric study on the interaction between norfloxacin (NFX) and metal ions (Al^{3+}, Mg^{2+} and Ca^{2+}). Two buffers of pH 3.6 and 8.8 were used. A shift in absorption maximum was observed and the stoichiometry of the complex was determined using the Job and molar ratio methods. The ratios were NFX:Al^{3+}, 2:1 and 3:1 for the NFX:Mg^{2+} complex. The formation of a complex with Al^{3+} enhanced the water solubility of the drug. Microbiological studies indicated decreased activity of norfloxacin in the presence of metal ions.

Introduction

Norfloxacin (NFX), a member of the 4-fluoroquinolone class of antimicrobial agents, is used clinically for the treatment of urinary tract infections. Earlier clinical studies had indicated decreased bioavailability of 4-quinolones in the presence of antacid preparations, particularly when administered concurrently or within a short period of time (Hoffken et al., 1985; Lode, 1988; Noyes and Polk, 1988; Nix et al., 1989). A consideration of the structure of the drugs indicates the possibility for the formation of drug-metal ion complexes. A study carried out on nalidixic acid confirmed the formation of such complexes (Nakano et al., 1978). However, further chemical investigations on 4-fluoroquinolones were not performed except on the bioavailability of the drugs (Nix et al., 1989).

In addition to drug interaction, Ratcliffe and Smith (1983) and Smith and Ratcliffe (1986) reported that the antimicrobial activity of 4-quinolones against *E. coli* is decreased in the presence of Mg^{2+}.

In this report, a spectrophotometric study was conducted in order to elucidate the interaction between metal ions and norfloxacin. In addition, a microbiological investigation was carried out to study the effect of metal ions on the antimicrobial activity of norfloxacin.

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Materials and Methods

Chemicals

Norfloxacin was generously provided by the Jordanian Pharmaceutical Manufacturing Co., Jordan, and was used as received. Aluminum nitrate, magnesium chloride, calcium chloride, sodium acetate, acetic acid, Tris and hydrochloric acid were of analytical reagent grade. Glass-distilled water was used for the preparation of solutions. Buffer solutions used were: 0.2 M acetate buffer for pH 3.6 and 0.5 M Tris buffer for pH 8.8.

Stock solutions

Norfloxacin, 0.1 M in ethanol, was used as a stock solution which was stable for at least 2 weeks. Further dilutions to 10^{-3} M were prepared using acetate buffer.

Metal ion solutions (0.1 M) were prepared in distilled water. Dilutions to 10^{-3} M were carried out using appropriate buffers.

Instrument

A Shimadzu UV-240 recording spectrophotometer equipped with 10 mm quartz cuvettes was used for measurements.

Bacterial strains

The strains used in this study were the following standard strains: *Pseudomonas aeruginosa* ATCC 27853; *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212.

Methods

Formation of norfloxacin-metal complex To a 5 ml aliquot of 10^{-3} M norfloxacin solution, 20 ml of 10^{-3} M stock solutions of metal ions (Al^{3+}, Mg^{2+} or Ca^{2+}) were added. The volume was made up to 100 ml with appropriate buffer: acetate buffer at pH 3.6 for Al^{3+} and Tris buffer at pH 8.8 for Mg^{2+} or Ca^{2+}. The UV absorption was recorded over the wavelength range 250–300 nm. All measurements were carried out at room temperature.

Stoichiometry of the complex was determined using both continuous variation and molar ratio methods (Ewing, 1985). Absorbance was measured at 290 nm for Al^{3+} and at 285 nm for Ca^{2+} or Mg^{2+} complexes, using a norfloxacin solution of equivalent concentration as a reference.

Solubility measurements Glass-stoppered test tubes containing an excess amount of norfloxacin powder in 0.2 M acetate buffer at pH 3.6, and variable concentrations of Al^{3+} (0.01–0.1 M) were immersed in a constant temperature shaking water bath set at 37°C. The mixtures were left to equilibrate for 7 days, after which they were filtered and diluted with acetate buffer (pH 3.6) for spectrophotometric measurements. Absorbance was recorded at the wavelength corresponding to the isosbestic point (281 nm) in the spectrum of norfloxacin at pH 3.6.

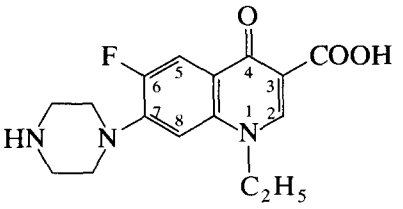
Measurement of MIC and MBC MICs were determined by the broth tube dilution method as described in the NCCLS approved performance standard M7-T (National Committee for Clinical Laboratories Standards, 1983) using Mueller-Hinton broth (Difco Laboratories, U.S.A.) without any divalent cation supplementation due to the nature of the study. The MIC was defined as the lowest concentration of the antimicrobial agent that inhibited the visible growth of the test organism after incubation. The MBC was obtained for each organism and was defined as the lowest concentration of the antimicrobial agent that resulted in 99.9% or greater killing of the initial inoculum.

The pH of the culture was measured before and after incubation, no change being observed. The pH values of the cultures were 6.0 for the control (no metal ions) and 7.0 for cultures containing Mg^{2+} and Ca^{2+}. A more acidic pH of 4.0 was needed to carry out the test on cultures to which Al^{3+} was added in order to avoid precipitation at higher pH values.

Results and Discussion

Chemically, norfloxacin (NFX) is an amphoteric molecule possessing two major ionizable groups, a basic piperazine amine and acidic carboxyl having dissociation constants of 6.3 and 8,

respectively (Jack, 1986):



The structure of NFX with a carbonyl adjacent to the carboxyl group allows for proposing the formation of a complex between metal ions and the drug. The possibility of such interactions was studied using spectrophotometric methods, where UV absorption was measured in the presence and absence of metal ions. Metal ions chosen for this study were those normally encountered in antacid preparations or in biological fluids, namely: aluminum, calcium and magnesium.

The measurements were carried out in two buffers, acetate buffer (pH 3.6) and Tris buffer (pH 8.8). The absorption maximum for the drug in acetate buffer was 277.5 nm. Addition of Al^{3+} shifted the maximum to 274 nm (Fig. 1a). The effect on the spectrum was negligible in the presence of Ca^{2+} and Mg^{2+}. However, under basic conditions, the drug alone absorbed at λ_{max} 273 nm which was shifted by the addition of Ca^{2+} and Mg^{2+} to 276 and 278 nm, respectively (Fig. 1b). Al^{3+} formed insoluble salts at pH 8.8.

Investigation of the effects of Al^{3+} concentration on complex formation at acidic pH showed that a 4-fold excess of the drug was required for the quantitative conversion of NFX into the complex. The latter is formed immediately, its absorbance remaining unchanged for 24 h.

The stoichiometric ratio of NFX to Al^{3+} was determined by applying Job's method of continuous variation. The measurements were carried out in acetate buffer (pH 3.6) at 290 nm using NFX as the reference standard, since under the reported experimental conditions, the drug absorbs within the same range as the complex. The plot constructed (Fig. 2a) displayed a maximum at a mole fraction of $X_{max} = 0.33$, which indicated the formation of a 2:1 complex of NFX:Al^{3+}.

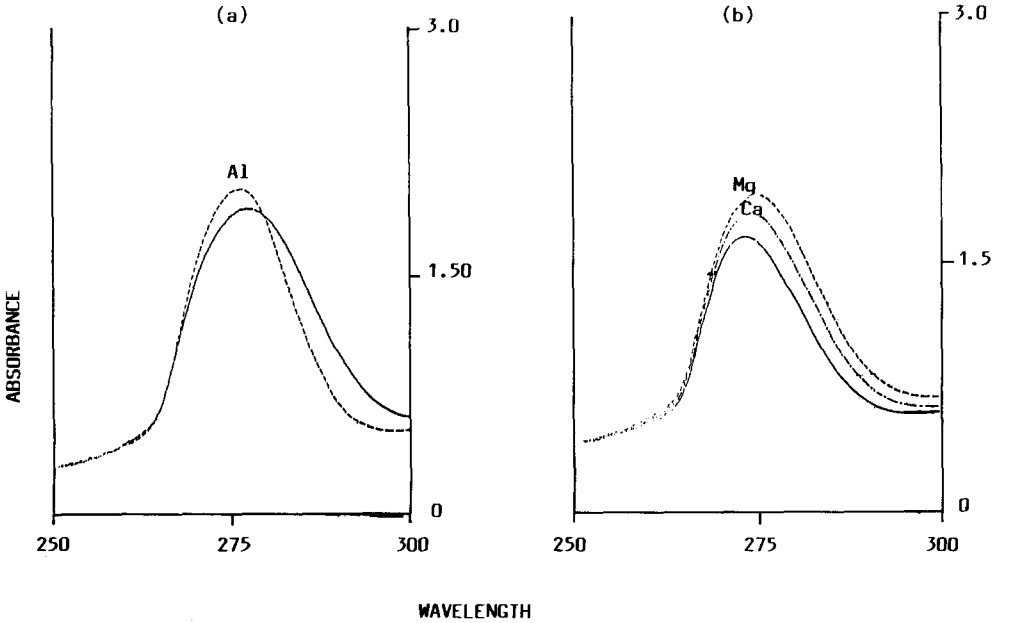


Fig. 1. (a) Absorption spectra of norfloxacin in acetate buffer, pH 3.6 alone (———) and in the presence of aluminum ions (------). (b) Absorption spectra of norfloxacin in Tris-buffer, pH 8.8 alone (———) and in the presence of calcium and magnesium ions (------).

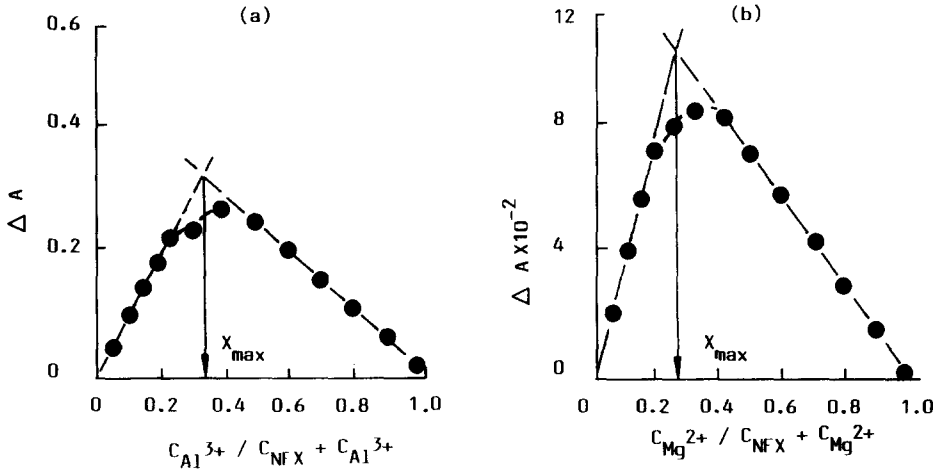


Fig. 2. Job plot of equimolar solutions of norfloxacin and metal ions. (a) [NFX] + [Al^{3+}] = 1×10^{-3} M, pH 3.6, measured at 290 nm; (b) [NFX] and [Mg^{2+}] = 1×10^{-3} M, pH 8.8, measured at 285 nm.

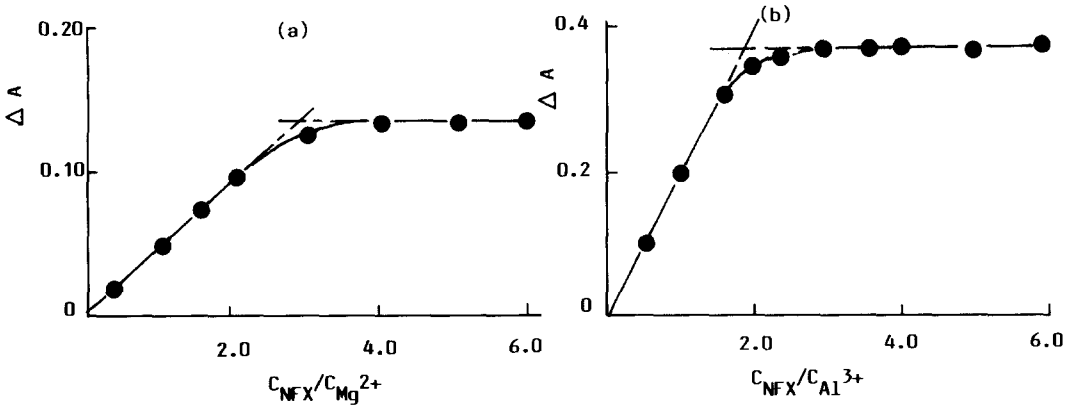


Fig. 3. Molar ratio plot. (a) For the complex of NFX (10^{-5} M) with Mg^{2+}, pH 8.8 measured at 285 nm; (b) for the complex of NFX (10^{-5} M) with Al^{3+}, pH 3.6 measured at 290 nm.

TABLE 1

Minimal inhibitory and minimal bactericidal concentrations in μg / ml for norfloxacin (NFX) alone and in the presence of Al^{3+}, Mg^{2+} and Ca^{2+}

	NFX		NFX + Al^{3+}		NFX + Ca^{2+}		NFX + Mg^{2+}	
	MIC ($\times 10^{-2}$)	MBC	MIC ($\times 10^{-2}$)	MBC	MIC ($\times 10^{-2}$)	MBC	MIC ($\times 10^{-2}$)	MBC
P. aeruginosa	15.6	1.25	62.5	2.5	31.2	2.5	125.0	10.0
S. aureus	0.06	0.019	0.03	0.019	1.9	0.62	3.9	0.15
E. faecalis	15.6	1.25	62.5	10.0	250.0	40.0	62.5	10.0

On the other hand, application of the same procedure to Mg^{2+} and NFX under basic conditions showed a maximum at a mole fraction $X_{max} = 0.25$ indicating the formation of a 3:1 complex of NFX:Mg^{2+} (Fig. 2b).

Further confirmation of the stoichiometry was obtained by the molar ratio method. Plots were constructed with intercepts at molar ratios of 2:1 and 3:1 for NFX:Al^{3+} and NFX:Mg^{2+} (Fig. 3b and a), respectively.

The effect of the proposed complexation on the solubility behaviour of the drug was investigated using Al^{3+} for the study, since it is the most commonly encountered ion in antacid preparations. Water solubility was determined at acidic pH. Gradual enhancement of NFX solubility with increase in Al^{3+} concentration was observed. When a plateau was reached at 0.1 M of Al^{3+}, the concentration of NFX in solution was 0.3 M as compared to 0.091 M in control solution lacking Al^{3+}, indicating an approx. 4-fold increase in water solubility.

The results of spectral and solubility studies are indicative of the formation of a drug:metal ion complex, possibly at carboxyl-carbonyl groups of the quinolone molecule. The complex formation could be responsible for the decreased absorption of NFX from the gastrointestinal tract probably through increasing the size of absorbed species.

In addition, the antimicrobial activity of NFX was determined in the presence of three metal ions, Al^{3+}, Mg^{2+} and Ca^{2+}, at the same concentration ratio required for complex formation, i.e., a 4:1 drug:metal ratio.

The measured minimal inhibitory concentrations (MIC) and the minimal bactericidal concentrations (MBC) for three standard bacterial strains are listed in Table 1.

The listed values are lower than those reported for NFX in the literature (Wolfson and Hooper, 1988) which could be attributed to the exclusion of divalent ions, as supplement to the broth used in carrying out the determination. The MIC value for *S. aureus* was unexpectedly lower in the presence of Al^{3+} which could be due to the low pH of the broth.

Considering the results reveals an increase in MIC and MBC values in the presence of metal ions which is consistent with earlier reports (Smith and Ratcliffe, 1986) using *E. coli* KL16 as the test organism for the effect of Mg^{2+} on the activity of 4-quinolones.

In conclusion, the present chemical and spectral investigations support the possibility of complex formation between metal ions and norfloxacin. The preliminary results show that such a complex may affect both the absorption and the antimicrobial activity of norfloxacin.

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