International Journal of Pharmaceutics, 93 (1993) 131–138

IJP 03046

Physicochemical properties of the fluoroquinolone antimicrobials VI. Effect of metal-ion complexation on octan-1-ol-water partitioning

Danna L. Ross [1], Steven K. Elkinton, Sheila R. Knaub and Christopher M. Riley

Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66045 (USA)

(Received 23 January 1992)
(Modified version received 8 July 1992)
(Accepted 16 September 1992)

Key words: Fluoroquinolone; Antimicrobial; Partition coefficient; Metal-ion complexation; Structural effects

Summary

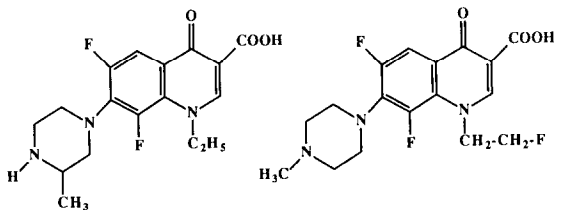
The effects of calcium chloride, magnesium chloride and aluminum chloride on the octan-1-ol-water partition coefficients of lomefloxacin and fleroxacin were studied at 25°C and pH 5.0 with an ionic strength of 0.35. Only part of the reduction of the partition coefficients of the quinolones following the addition of those salts could be attributed to complexation with the metal ions. The addition of metal ions also appeared to decrease the partitioning of ion-pairs formed between the cationic forms of the quinolones and the chloride and acetate ions present in the buffer solution.

Introduction

One of the most widely quoted and frequently investigated drug interactions of the fluoroquinolones arises from their apparent interactions with antacids and other drugs, such as ranitidine that modify the intestinal pH (Flor et al., 1985, 1990; Hoffken et al., 1985a,b, 1988; Frank et al., 1986; Lener et al., 1987; Schentag et al., 1988; Frost et al., 1989a,b; Grasela et al., 1989; Nix et al., 1989a,b; Polk, 1989; Polk et al., 1989; Brouwers et al., 1990). Co-administration of the fluoroquinolones with antacids has also been implicated in a reduction in antimicrobial activity in urine (Barbhaiya et al., 1982; Ratcliffe et al., 1983; Pohlod et al., 1984; Kumada et al., 1985; Smith et al., 1985; Blaser et al., 1988; Smith et al., 1988; Gurdal et al., 1990; Perez-Giraldo et al., 1990). This reduction in activity has been attributed to the high concentration of magnesium in urine. This theory was supported by the observations that the activity of the fluoroquinolones is lower in urine than in buffer and that addition of magnesium and other cations to urine further reduces the antimicrobial activity (Ratcliffe and Smith, 1983; Blaser et al., 1985; Smith and Rat-

Correspondence to: C.M. Riley, Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66045, U.S.A.

[1] *Present address:* 3M Pharmaceuticals, 3M Center, St. Paul, MN 55144 U.S.A.



Lomefloxacin Fleroxacin

Fig. 1. Structures of lomefloxacin and fleroxacin.

cliffe, 1985; Blaser and Luthy, 1988; Gurdal et al., 1990; Perez-Giraldo et al., 1990).

In a recent paper, Ross and Riley (1992b) have shown that complexation of lomefloxacin with Ca^{2+}, Mg^{2+}, Fe^{3+} or Al^{3+} produces complexes with increased aqueous solubility compared with lomefloxacin itself. In contrast, complexation with Bi^{3+} substantially reduced the solubility of lomefloxacin. Those results suggests that decreased bioavailability and reduced antimicrobial activity in urine following administration of preparations containing Ca^{2+}, Mg^{2+}, Fe^{3+} or Al^{3+} are due to reduced hydrophobicity rather than precipitation of the drug in the gastro-intestinal tract. To test this hypothesis, the effects of complexation with Ca^{2+}, Mg^{2+}, and Al^{3+} on the partitioning of lomefloxacin and fleroxacin (Fig. 1) from water into octan-1-ol have been investigated. Previously this group has reported on the effects of pH on the octan-1-ol-water partition coefficients of the fluoroquinolones (Ross et al., 1992).

Materials and Methods

Materials

Samples of fleroxacin and lomefloxacin mesylate were obtained from Hoffmann-LaRoche Inc., Nutley, NJ and G.D. Searle and Co., Skokie, IL, respectively. All solvents were HPLC grade and obtained from commercial sources. All buffer components and metal salts (in their chloride forms) were ACS reagent grade or better and obtained from commercial sources. All other chemicals were reagent grade obtained from commercial sources. Water was purified in a Milli-Q Water System (Millipore Corp., Bedford, MA) and stored in glass containers until use.

Dithizone test

Glassware was washed with nitric acid and rinsed with metal-free water before use to eliminate any trace metal contaminants and all buffers were checked for the presence of heavy metal ions using the dithizone test (Stout and Arnon, 1939).

Apparatus

All pH measurements were made using an Orion SA 520 pH meter (Orion Research, Inc., Boston, MA) and a Tiny Combination pH electrode (Microelectrodes, Inc., Londonderry, NH) or a calomel pH combination glass electrode (Markson, Phoenix, AZ). Samples were assayed for quinolone content by LC using either a Waters chromatographic pump (Waters Associates, Milford MA), or a Beckman Model 110A pump (Beckman Instruments, Inc., Fullerton, CA), a Waters Model U6K injector or an Altex 210 injector (Beckman Instruments, Inc.) fitted with a 20 μl loop, a Waters Model 440 absorbance detector with a 280 nm filter, a Spectroflow 757 Absorbance Detector (Kratos Analytical, Ramsey, NJ), or a Unimetric FS 970 L.C. Fluorometer (Unimetrics Corporation, Ramsey, NJ) and an OmniScribe Recorder (Houston Instrument, Austin, TX).

Chromatographic conditions

The LC assays were conducted using an MOS Hypersil (C8) reversed-phase column (5 μm, 15 cm $\times$ 4.6 mm, i.d.) and either UV detection at 280 nm or fluorescence detection (excitation, 288 nm; emission, 418 nm cutoff filter). The mobile phase was THF-acetonitrile-H_3PO_4 (100 mM)-triethylamine-water (10 : 30 : 10 : 0.03 : qs 100) with a flow rate of 1.5 ml min^{-1}. All injections were made in duplicate.

Partition coefficient determinations

The partition coefficient of lomefloxacin in the presence of Ca^{2+}, Mg^{2+}, and Al^{3+} was studied at 25°C. A series of solutions were prepared in pH 5 buffer (acetate, 0.1 M, μ = 0.35; equilibrated with

octan-1-ol) in which the concentration of metal ion was increased incrementally while the drug concentration was held constant at 10 μg ml^{-1}. Equal volumes of the aqueous solution and octan-1-ol (equilibrated with acetate buffer) were mixed, protected from light by wrapping the vials in aluminum foil, and agitated for 24 h in a water bath at 25°C. After equilibrium was achieved, the samples were centrifuged and the two phases were separated. The aqueous layer was diluted with mobile phase and assayed by LC. 1 ml of the octanol layer was back-extracted with 5 ml of mobile phase by vortexing for 1 min. The mobile phase fraction was injected directly into the chromatograph to determine the concentration in the octanol phase. The recovery of each quinolone from octan-1-ol into mobile phase was 100% under all conditions studied. All partition coefficient determinations were conducted in triplicate.

Results and Discussion

Effects of magnesium or calcium on partitioning

The presence of Mg^{2+}, or Ca^{2+} resulted in a decrease in the apparent partition coefficient of lomefloxacin (Fig. 2a). At pH values equal to or less than 7 the second dissociation constant of the fluoroquinolones corresponding to deprotonated of the 4′-piperazinyl nitrogen may be ignored and the relationship between the apparent partition coefficient (D), the dissociation constants (K_{a_1}), and K_{11} can be described by:

$$D = \frac{C_{tot,o}}{C_{tot,w}} = K_D\left(\frac{K_{a_1}}{[H^+] + K_{a_1} + K_{11}[M]}\right) \quad (1)$$

where K_D is the thermodynamic distribution coefficient of the zwitterionic form, $HQ^{\pm}$, and [M] denotes the concentration of metal ions. Eqn 1 may be rearranged to give Eqn 2 which predicts a linear relationship between the function K_D/D and [M].

$$\frac{K_D}{D} = \left(1 + \frac{[H^+]}{K_{a_1}}\right) + K_{11}[M] \quad (2)$$

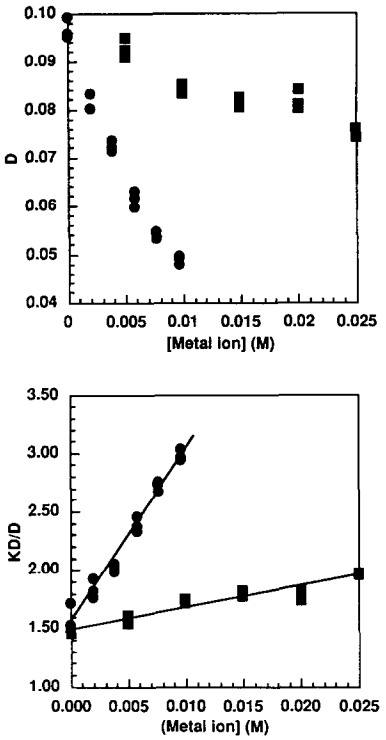


Fig. 2. Lomefloxacin apparent partition coefficient (D) between octan-1-ol and water (25°C) as a function of metal ion concentration plotted according to Eqns 1 (upper) and 2 (lower): Ca^{2+} (■) (pH 5.2, μ = 0.35 with NaCl) and Mg^{2+} (●) (pH 5.3, μ = 0.35 with NaCl). The points are experimental and the lines are the best fit lines obtained by least-squares linear regression analysis of the data according to Eqn 2.

For Eqns 1 and 2 to apply, it is necessary to assume that the metal-ion complex does not partition into the octanol phase and that complexation with the zwitterionic form ($HQ^{\pm}$) of the drug is much greater than the complexation with the cationic species (H_2Q^+). The second assumption has been verified in the preceding article (Ross and Riley, 1993). That assumption cannot be made for the aluminum complexes which form higher order complexes (Ross and Riley, 1992b). Aluminum ions also bind to each of the Bjerrum species of the fluoroquinolones.

Fig. 2b shows the linear relationships between the values of K_D/D for lomefloxacin and the concentration of added magnesium or calcium ions. Least-squares linear regression of the data (Eqn 2, Fig. 2b) gave the following values for the

TABLE 1

Equilibria constants for the ionization (K_a), metal-ion complexation (K_{11}) and octan-1-ol-water partitioning (K_D) of lomefloxacin and temafloxacin at 25°C ($\mu = 0.35$)

Parameter	Analytical method	Value		Ref.
		Lomefloxacin	Flerofloxacin	
pK_{a_1}	UV spectroscopy	5.82	5.46	a
pK_{a_2}	UV spectroscopy	9.30	8.10	a
K_{11} (Mg^{2+}) (M^{-1})	fluorescence	432	465	b
K_{11} (Mg^{2+}) (M^{-1})	partitioning	151	592	c
K_{11} (Ca^{2+}) (M^{-1})	fluorescence	69	not done	b
K_{11} (Ca^{2+}) (M^{-1})	partitioning	17.8	not done	c
K_D	partitioning	0.146	0.294	d

[a] Ross and Riley (1990).
[b] Ross and Riley (1992b).
[c] This study.
[d] Ross et al. (1992).

association constants of the lomefloxacin : magnesium and lomefloxacin : calcium complexes: K_{11} (Mg^{2+}) 151.1 M^{-1}, K_{11} (Ca^{2+}) 17.8 M^{-1}. These binding constants were substantially less than those determined by the fluorescence (Ross and Riley, 1993) (Table 1). The difference between the association constants obtained by partitioning and those obtained previously indicates the presence of additional equilibria not accounted for by Eqns 1 and 2. Evidence for additional equilibria was obtained previously by Ross et al. (1992) who found that the value of the apparent partition coefficient of lomefloxacin at pH 5 was greater than predicted from the value of K_d obtained at pH 7. Although the partitioning experiments were conducted at higher drug concentrations than that of the fluorescence binding studies, there should be no influence of concentration on binding if 1 : 1 complexes are the only species present. Also, solubility studies (Ross and Riley, 1992b) of lomefloxacin in the presence of metal ions resulted in comparable binding constants to those determined in the fluorescence studies confirming that drug concentration does not affect binding to Ca^{2+} or Mg^{2+} and hence could not explain the smaller binding constants obtained in the partitioning studies. The ionic strength was held constant at 0.35 for both the partitioning experiments and the fluorescence experiments. Buffer effects could also be discounted because the same buffer was used for the partitioning and fluorescence experiments.

Additional factors that might contribute to the observed partitioning of lomefloxacin in the presence of metal ions included the influence of dissolved octan-1-ol in the aqueous phase on the metal-ion complexation, the presence of lomefloxacin association species or micelles and the formation of ion-pairs between the cationic form of the drug (H_2Q^+) and anions in the aqueous phase.

Influence on octan-1-ol on the binding constants

To ascertain if the binding of drug to metal ion changed in the presence of octanol, the fluorescence binding studies (Ross and Riley, 1993) were repeated for lomefloxacin and Mg^{2+} in buffer and octanol-saturated buffer. The resulting curves were superimposable (data not shown) indicating that the presence of dissolved octanol did not change the binding of drug to the metal ion.

Possible effects of micelle formation on partitioning

A possible explanation for the decrease in partitioning observed is the formation of a micellar drug phase in the aqueous medium. The ability of the complexed drug to form micelles may be greater than that of the uncomplexed drug because of the net positive charge on the complexed drug. With surfactants it has been found that the larger the charge-bearing atom in the polar head, the greater the aggregation number of the micelle (Anacker, 1978). Also, aliphatic alcohols have been found to decrease the critical micelle concentration of anionic and cationic surfactants (Tomlinson et al., 1978). The combination of a larger charge bearing atom on the complex and the presence of small amounts of octanol in the aqueous phase may entrance the ability for micelles to form.

Prior to the formation of micelles, amphiphilic molecules form a monolayer at the water-air interface which leads to a reduction in surface

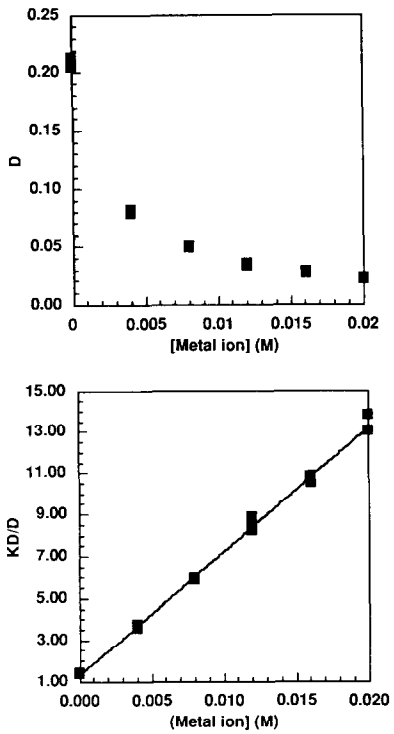


Fig. 3. Fleroxacin apparent partition coefficient (D) between octan-1-ol and water (25°C) as a function of [Mg^{2+}] (M) (pH 7.2 $\mu = 0.35$ with NaCl) plotted according to Eqns 1 (upper) and 2 (lower). The points are experimental and the line is the best fit line obtained by least-squares linear regression analysis of the data according to Eqn 2.

tension (Langmuir, 1917; Preston, 1948; Martin et al., 1983). Therefore, to determine if micelle formation in the presence of the metal ion was occurring, the surface tension of two solutions, one containing metal ion and one without was studied as a function of drug concentration using a DuNuoy ring-pull apparatus with a platinum-iridium ring. These experiments were conducted with octanol-saturated buffer at drug and metal concentrations similar to the partitioning experiments. The surface tensions in the presence and absence of Mg^{2+} were equal indicating that micell formation did not account for the decreased partitioning observed.

Effects of ion-pair formation

Previously it was shown that the partition coefficient of lomefloxacin at pH 5 was higher than predicted by theory and this was attributed to ion-pair formation between the cationic species of lomefloxacin and the acetate or chloride ions present in the aqueous phase (A^-) (Ross et al., 1992). The relationship between the apparent partition coefficient, D', of the ion-pair, $H_2Q^{\delta+}$, $A^{\delta+}$, and the concentration of the anion in the aqueous phase is given by:

$$D' = K'_D[A^-] \quad (3)$$

where K'_D is the ion-pair distribution constant. Thus, partitioning of the fluoroquinolone into the organic phase would increase with increasing concentration of the anion, A, in the aqueous phase. This process could be expected to compete with the metal-ion complexation and thus reduce the values of the apparent association constants (K_{11}) measured by partitioning.

To determine if decreased ion-pair formation could account for the difference in the apparent binding constant obtained by fluorescence and partitioning, the partition coefficient of fleroxacin was determined at pH 7 with changing Mg^{2+} concentrations. Fleroxacin was chosen for this study because it is a tertiary amine and ion-pairing was not observed with this compound in acetate buffer at pH 5 (Ross et al., 1992). Also fleroxacin has a pK_{a_1} of 5.46 and therefore, at pH 7 the fraction of the total present as the cationic species would be negligible and thus ion-pair formation would be expected to be inconsequential. Fig. 4a shows the apparent partition coefficient of fleroxacin between octa-1-ol

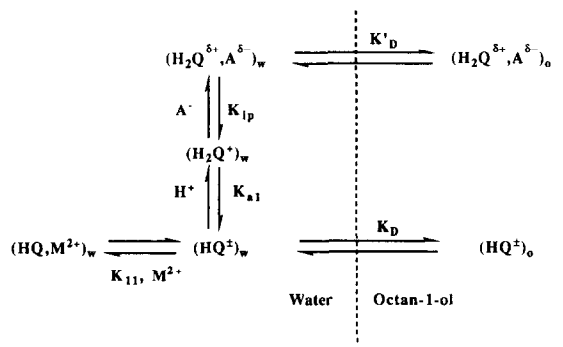


Fig. 4. Important equilibria involved in the partitioning of fluoroquinolones between octan-1-ol and water in the presence of magnesium or calcium ions.

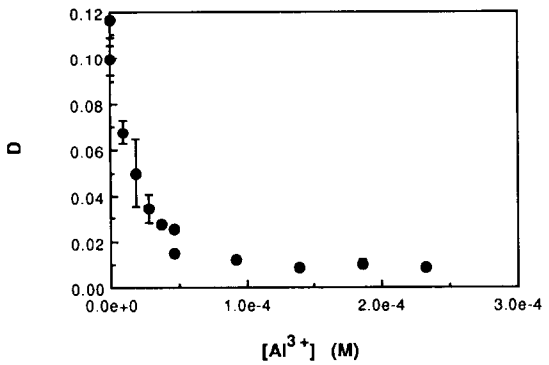


Fig. 5. Lomefloxacin apparent partition coefficient (D) between octan-1-ol and water (25°C, pH 5.1, $\mu = 0.35$ with NaCl) as a function of $[Al^{3+}]$.

and water as a function of Mg^{2+} concentration, and Fig. 4b shows the same data plotted according to Eqn 2. In this case the value K_{11} obtained by least squares linear regression analysis of 592 M^{-1} was in good agreement with the values of 465 M^{-1} obtained by fluorescence. These data support the hypothesis that in acidic solutions those fluoroquinolones with a tertiary amine at the 4′-position on the piperizinyl ring (e.g., fleroxacin) partition into an organic phase in the unionized form. On the other hand those fluoroquinolones containing a secondary amine at the 4′-position on the piperizinyl ring (e.g., lomefloxacin) may also partition into an organic phase as ion-pairs with anions present in the aqueous phase. Fig. 4 shows the various equilibria contributing to the partitioning of the fluoroquinolones in the presence of magnesium or calcium ions below pH 7.0.

Effects of aluminum ions on the partitioning

Fig. 5 shows that the partition coefficient of lomefloxacin decreased with increasing concentration of aluminum chloride. As with magnesium and calcium ion the partition coefficients determined at concentrations of Al^{3+} at or above 1×10^{-4} M are below those predicted by theory in the absence of both metal ions and ion-pairing. The fluoroquinolones forms 3:1 (drug:metal) complexes with aluminum and all three Bjerum species appear to bind to the metal ion (Ross and Riley, 1992b). Therefore, the association of the fluoroquinolones with aluminum is more complex than the association with calcium and magnesium and did not lend itself to a simple mathematical treatment.

Conclusions

The addition of magnesium, calcium or aluminium ions (as their chloride salts) results in a decrease in the octan-1-ol-water partition coefficients of the model fluoroquinolones, lomefloxacin and fleroxacin. The mechanism appears to involve decreased lipophilicity of the fluoroquinolones following complexation with metal ions. Thus the decrease in bioavailability and the reduced antimicrobial activity seen in the presence of metal ions may be explained on the basis of a decrease in lipophilicity of the complex compared with the free drug. Of course, these results do not preclude metal-ion facilitated transport across cell membranes which may occur in biological systems.

Acknowledgements

This work was supported by a grant from G.D. Searle, graduate fellowships for D.L.R. and S.R.K. from the American Foundation for Pharmaceutical Education (AFPE) and an undergraduate fellowship for S.K.E. from the Pharmaceutical Manufacturers Association (PMA). The authors are grateful to Drs Howard Lambert (The Nutrasweet Company), Patricia Frank (G.D. Searle) and Arnie Repta (Interx Corporation) for their helpful discussions.

References

Anacker, E., Electrolyte effect on micellization In Mittal, K. (Ed.), *Solution Chemistry of Surfactants*, Plenum, New York, 1978, pp. 247–265.

Barbhaiya, R., Gerber, A., Craig, W. and Welling, P., Influence of urinary pH on the pharmacokinetics of cinoxacin in humans and on antibacterial activity in vitro. *Antimicrob. Agents Chemother.*, 21 (1982) 472–480.

Blaser, J., Dudley, M., Gilbert, D. and Zinner, S., Influence of media and method on the in vitro susceptibility of Pseudomonas aeruginosa and other bacteria to ciprofloxacin and enoxacin. In Ishigami, J. (Ed.), *Recent Advances in Chemotherapy, Antimicrobial, Sect. 2, Proc. 14th Int. Congr. Chemother., Kyoto, University of Tokyo Press*, Tokyo, 1985, pp. 1547–1548.

Blaser, J. and Luthy, R., Comparative study on antagonistic effects of low pH and cation supplementation on in-vitro activity of quinolones and aminoglycosides against Pseudomonas aeruginosa. *J. Antimicrob. Chemother.*, 22 (1988) 15–22.

Brouwers, J., Van der Kam, H., Sijtsma, J. and Proost, J., Decreased ciprofloxacin absorption with concomitant administration of ferrous fumarate. *Pharm. Weekbl. Sci. Ed.*, 12 (1990) 182–183.

Flor, S., Guay, D., Opsahl, J., Tack, K. and Matzke, G., Effects of magnesium-aluminum hydroxide and calcium carbonate antacids on bioavailability of ofloxacin. *Antimicrob. Agents Chemother.*, 34 (1990) 2436–2438.

Flor, S., Weintraub, H., Marriott, T., Freidmann, N. and Beals, B., Pharmacokinetics of ofloxacin in humans after various single oral doses. In Ishigami, J. (Ed.), *Recent Adv. Chemother., Proc. 14th Int. Congr. Chemother., Antimicrobial, Sect. 2, Kyoto*, University of Tokyo Press, Tokyo, 1985, pp. 1783–1784.

Frank, W., Peace, K., Watson, J., Szego, P., Braverman, A., Mico, B. and Dickson, B., The effect of single intravenous doses of cimetidine or ranitidine on gastric secretion. *Clin. Pharmacol. Ther.*, 40 (1986) 665–672.

Frost, R., Carlson, J., Dietz, A., Heyd, A. and Lettieri, J., Ciprofloxacin pharmacokinetics after a standard or high-fat/high-calcium breakfast. *J. Clin. Pharmacol.*, 29 (1989a) 953–955.

Frost, R., Lettieri, J., Noe, A., Shamblen, E. and Lasseter, K., Effect of aluminum hydroxide and calcium carbonate antacids on ciprofloxacin bioavailability. *Clin. Pharmacol. Ther.*, 45 (1989b) 165.

Grasela Jr., T., Schentag, J., Sedman, A., Wilton, J., Thomas, D., Schultz, R., Lebsack, M. and Kinkel, A., Inhibition of enoxacin absorption by antacids or ranitidine. *Antimicrob. Agent Chemother.*, 33 (1989) 615–617.

Gurdal, H., Tulunay, F. and Altay, G., Post antibiotic effect of ofloxacin and the activity of Mg^{++}. *J. Antimicrob. Chemother.*, 26 (1990) 291–292.

Hoffken, G., Lode, H., Prinzing, C., Borner, K. and Koeppe, P., Pharmacokinetics of ciprofloxacin after oral and parenteral administration. *Antimicrob. Agents Chemother.*, 27 (1985a) 375–379.

Hoffken, G., Lode, H., Wiley, R., Glatzel, P., Borner, K. and Koeppe, P., Interactions on the gastrointestinal absorption of ciprofloxacin. In Ishigami, J. (Ed.), *Recent Advances in Chemotherapy, Antimicrobial, Sect. 2, Proc. 14th Int. Congr. Chemother. Kyoto,* University of Tokyo Press, Tokyo, 1985b, pp. 1606–1607.

Hoffken, G., Lode, H., Wiley, R., Glatzel, T., Sievers, D., Olschewski, T., Borner, K. and Koeppe, T., Pharmacokinetics and bioavailability of ciprofloxacin and ofloxacin: effect of food and antacid intake. *Rev. Infect. Dis.*, 10 (Suppl. 1) (1988) S138–S139.

Kumada, T., Ooi, S., Totsuka, K. and Shimizu, K., Antimicrobial activity of quinolone antibiotics in urine. In J. Ishigami (Ed.), *Recent Advances in Chemotherapy, Antimicrobial, Sect. 2, Proc. 14th Int. Congr. Chemother., Kyoto*, University of Tokyo Press, 1985, pp. 1881–1882.

Langmuir, I., The constitution and fundamental properties of solids and liquids. II. Liquids. *J. Am. Chem. Soc.*, 39 (1917) 1848–1906.

Lener, M., Watson, A., Krol, G., Goldstein, H., Frost, W., Lettieri, J. and Schentag, J., Antacid inhibition of ciprofloxacin in normal volunteers. *Pharm. Res.*, 4 (1987) S-79.

Martin, A., Swarbrick, J. and Cammarata, A. *Physical Pharmacy*, 3rd Edn, Lea and Febiger, Philadelphia, 1983.

Nix, D., Watson, W., Lener, M., Frost, R., Krol, G., Goldstein, H., Lettieri, J. and Schentag, J., Effects of aluminum and magnesium antacids and ranitidine on the absorption of ciprofloxacin. *Clin. Pharmacol. Ther.*, 46 (1989a) 700–705.

Nix, D., Wilton, J., Schentag, J., Parpia, S., Norman, A. and Goldstein, H., Inhibition of norfloxacin absorption by antacids and sucralfate. *Rev. Infect. Dis.*, 11 (Suppl. 5) (1989b) S1096.

Perez-Giraldo, C., Hurtado, C. Moran, F. and Blanco, M., The influence of magnesium on ofloxacin activity against different growth phases of *Escherichia coli*. *J. Antimicrob. Chemother.*, 25 (1990) 1021–1026.

Pohlod, D.J. and Saravolatz, L.D., In vitro susceptibilities of 393 recent clinical isolated to WIN 49375, cefotaxime, tobramycin, and piperacillin. *Antimicrob. Agents Chemother.*, 25 (1984) 377–379.

Polk, R., Drug-drug interactions with ciprofloxacin and other fluoroquinolones. *Am. J. Med.*, 87 (Suppl. 5A) (1989) 76S–81S.

Polk, R., Healy, D., Sahai, J., Drwal, L. and Racht, E., Effect of ferrous sulfate and multivitamins with zinc on absorption of ciprofloxacin in normal volunteers. *Antimicrob. Agents Chemother.*, 33 (1989) 1841–1844.

Preston, W., Some correlating principles of detergent action. *J. Phys. Coll. Chem.*, 52 (1948) 84–97.

Ratcliffe, N. and Smith, J., Effects of magnesium on the activity of 4-quinolone antimicrobial agents. *J. Pharm. Pharmacol.*, 35 (1983) 61P.

Ross, D.L. and Riley, C.M., Aqueous solubilities of some variously substituted quinolone antimicrobials. *Int. J. Pharm.*, 63 (1990) 237–250.

Ross, D.L. and Riley, C.M., Physicochemical properties of the fluoroquinolone antimicrobials. II: Acid ionization constants and their relationship to structure. *Int. J. Pharm.*, 83 (1992a) 267–272.

Ross, D.L. and Riley, C.M., Physicochemical properties of the fluoroquinolone antimicrobials. III: Complexation of

lomefloxacin with various metal ions and the effect of metal ion complexation on aqueous solubility. *Int. J. Pharm.*, 87 (1992b) 203–213.

Ross, D.L. and Riley, C.M., Physicochemical properties of the fluoroquinolone antimicrobials. V: Effect of fluoroquinolone structure and pH on the complexation of various quinolones with magnesium and calcium. *Int. J. Pharm.*, 93 (1993) 121–129.

Ross, D.L., Elkinton, S. and Riley, C.M., Physicochemical properties of the fluoroquinolone antimicrobials. IV: 1-Octanol-water partition coefficients and their relationships to structure. *Int. J. Pharm.*, 99 (1992) 379–389.

Schentag, J., Watson, W., Nix, D., Sedman, A., Frost, R. and Letteri, J., Time dependent interactions between antacids and quinolone antibiotics. *Clin. Pharmacol. Ther.*, 43 (1988) 135.

Stout, P. and Arnon, D., Experimental methods for the study of the role of copper, managanese, and zinc in the nutrition of higher plants. *Am. J. Bot.*, 26 (1939) 144–149.

Smith, J. and Lewin, C., Chemistry and mechanisms of action of the quinolone antibacterials In Andriole, V. (Edn.), *The Quinolones*, Academic Press, San Diego, 1988, pp. 23–82.

Smith, J. and Ratcliffe, N., Effect of pH and magnesium on the in vitro activity of ciprofloxacin. In Neu, H. and Weuta, H. (Eds.), *First International Ciprofloxacin Workshop*, Excerpta Medica, Leverkusen, 1985.

Tomlinson, E., Guveli, D., Davis, S. and Kayes, J., Anomalous behaviour of aromatic alcohols on the critical micelle concentrations of cationic surfactants In Mittal, K. Ed., *Solution Chemistry of Surfactants*, Plenum, New York, 1978, pp. 355–366.