CHAPTER II

Literature Review
ANTIBIOTIC DRUGS

(1) NALIDIXIC ACID

Its Chemical name is (1-ethyl-7-methyl-4-oxo-1, 4-dihydro -1, 8-naphthyridine-3- carboxylic acid) has the following structure.

![Chemical structure of Nalidixic Acid]

Fig. (1.1) : NALIDIXIC ACID

Its molecular formula is C₁₂H₁₉N₃O₃ and molecular weight is 232.24. It is almost pale yellow, odourless, crystalline powder. Insoluble in water, slightly soluble in alcohol, soluble 1 in 300 of acetone, 1 in 25 of chloroform, soluble in solution of alkali hydroxides and carbonate solution. It is stored in light resistant containers.¹

It inhibits gram negative microbes including Escherichia. coli, klebsiella aerogenes, K.pheumoniae, S. typhi, Shigella flexneri and proteus species. Gram positive bacteria are usually resistant. Its antimicrobial activity is not significantly affected by difference in urinary pH. It is suggested that nalidixic acid interferes in the conversion of intermediate size DNA fragments into bigger DNA molecules, in the presence of competent RNA and protein synthesis.²

It is readily absorbed from the gastrointestinal tract [95%], remainder passes out in stool. Serum levels of 20 to 50 mcg/ml are attained 2 hour after a single oral dose of lg. In blood, nalidixic acid is 93% protein bound and hydroxy nalidixic acid, an active metabolite of nalidixic acid is 63% protein-bound. The urine contains 50-500 mcg/ml of active antibacterial principle.³
Both, nalidixic acid and its metabolite, are rapidly conjugated in the liver with half life of 8 hour. The prostate dose not attain therapeutic drug levels. It interacts with warfarin type drugs, introfurantoin and probenecid and gives false positive tests for hyperglycaemia and 17-ketosteroids. Side effects include nausea, vomiting of other gastrointestinal disturbances, drowsiness and weakness. "R"-factor resistance to nalidixic acid has never been demonstrated. It is used in treatment of urinary tract infection due to gram negative bacteria other than Pseudomonas species. It has also been used in treatment of bacterial dysentery, infections which have not responded to antibiotics and sulphonamides.

(2) NORFLOXACIN

Its chemical name is [3-quinolinecarboxylic acid, 1-ethyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-]. Its molecular formula C₁₆ H₁₈ F N₃ O₃ and molecular weight is 319.34. It is a white to pale yellow crystalline powder melting about 221°. It is hygroscopic and forms a hemihydrate air. It is very slightly soluble is water, methanol or alcohol; freely soluble in glacial acetic acid.

It is a potent drug capable of destroying bacteria like Pseudomonas aeruginosa, Escherichia coli, Klebsiella and multidrug resistant bacteria including methicillin resistant Staphylococcus aureus. Thus with the advent of fluoroquinlones a breed of drugs unrelated to the conventional and newer antibacterial agents has opened vistas to overcome the power of resistance development by bacteria.

It is used in the treatment of urinary tract infections [U.T.I] including cystitis, urethritis, pyelonephritis and prostatis, it is active against Pseudomonas aeruginosa and Prophylactic against traveller's diarrhoea.
[3] CIPROFLOXACIN

Its chemical name is [3-quinolinecarboxylic acid, 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)] - has the following structure.⁶

[Image of Ciprofloxacin molecule]

Fig. (1.3) : CIPROFLOXACIN

Its molecular formula is $C_{17}H_{18}F_N_3O_3$ and molecular weight is 331.37. It is white to off white crystalline powder and freely soluble in water. It is approved for use in the treatment of bone and joint infections caused by Enterobacter cloace, Pseudomonas aeruginosa and Serratia marcescens; infections diarrhea by Campylobacter jejuni or Shigella flexneri; lower respiratory tract infections by Enterocloacae, Escherichia coli; skin infections by Citrobacter freundil, Enteroc cloacae; and urinary tract infections [U.T.I.] by citrobactor diversus or freundii, Escherichia coli. It is a second choice drug in all of the above.
It is the drug of choice for the treatment of infections by *Campylobacter jejuni*. In addition, it is an unlabeled but authoritatively alternative drug for the treatment of *Gonorrhea* and *Salmonella* and *Yersinia* infections.

These drugs [Norfloxacin and Ciprofloxacain] are chemically related to nalidixic acid and are called fluoroquinolones because of the fluorine in their chemical structure. Like nalidixic acid, they are effective orally. Fluoroquinolones are rapidly bacteridal and, like nalidixic acid, probably act by inhibiting bacterial DNA replication. Bacteria can develop resistance to fluoroquinolones by mutation. They have relatively broad spectrum of action and are effective against gram positive and gram negative organisms.

[4] CLOXACILLIN SODIUM

Its chemical name is [2S-(2α, 5α; 6β)]-4-Thia-1-azabicyclo [3, 2, 0] heptane-2-carboxylic acid, 6-[[3-(2-chlorophenyl)-5-methyl-4-isoxazolyl] carbonyl] amino]-3, 3-dimethyl-7-oxo-, monosodium salt, monohydrate, has the following structure.  

![Chemical Structure of Cloxacillin Sodium](image)

**Fig. (1.4) : CLOXACILLIN SODIUM**

Its molecular formula C_{19}H_{17}ClN_{3}NaO_{5}S·H_{2}O and molecular weight is 475.88. It is a white, odourless, crystalline powder having a bitter taste, melts between 170°-173° C. Freely soluble in water, soluble in alcohol; slightly soluble in acetone and chloroform.
This penicillin is effective in the treatment of infections due to penicillinase producing *Staphylococci*. The drug shares a first-choice status with other penicillinase-resistant penicillins in the treatment of *Staphylococcus* infections caused by penicillin G-resistant strains. However, it is less active than penicillin G against nonpenicillinase-producing bacteria, especially *Streptococci*. It is not effective against gram negative organisms. Consequently, its use should be limited to treating infections caused by penicillinase-producing susceptible microorganisms which are resistant to penicillin G. By thus limiting its use, the development of resistance to cloxacillin is discouraged.6

**REVIEW OF THE RELEVANT LITERATURE ON THEIR METALLIC COMPLEXES**

Survey of the literature reveals, that almost negligible has been done as for as the metal complexes of, nalidixic acid, norfloxacin, ciprofloxacin and cloxacillin sodium. Nalidixic acid belong to quinolone group. Where as norfloxacin and ciprofloxacin are fluoroquinolones. Cloxacillin sodium is an antibiotic of penicillin group. All the four drugs studied are antibiotics.

There are many studies on the antimicrobial activity in-vitro of norfloxacin and some have been the subject of detailed reviews.11-12 Resistance can be introduced13 as can cross resistance between the 4-quinolones, although it has been considered unlikely that such resistance would diminish any clinical effect since the increased MICs are still within achievable concentration in-vitro.14 Single oral dose of norfloxacin therapeutic concentration of drug are rapidly achieved in serum and high reactions of tissue to serum concentration have been observed in both human renal and prostatic tissue. Approximately 33% of the absorbed drug is excreted unchanged in urine. The primary site of norfloxacin metabolism is liver. The activity of norfloxacin also appears to be influenced in pH with its activity
being diminished in acid media. Sensitive gram negative organisms include *Escherichia coli, Citrobacter, Entrobacter, Klebsiella proteus, Salmonella, Shigella* and *Yersinia spp.* Pseudomonas aeruginosa is susceptible as are other *Pseudomonas spp*, but to a lesser degree.

Administration of norfloxacin by mouth readily produces urinary concentration that are bactericidal to most urinary tract pathogens, including pseudomonas aeruginosa. It is therefore used in the treatment of urinary tract infections. It has been tried in ganococcal arthritasis as well as bacterial gastroenteritis.

The fluoroquinolones are a new class of antimicrobial agents that are now widely prescribed for a number of bacterial infections. Because of their complex pharmacokinetics, there is a potential for several types of drug interactions. Currently, only two drug interactions have been well studied. These involve a decrease in absorption when fluoroquinolones are given in combination with multivalent metal cations and an inhibition in the metabolism of methylxanthines by fluoroquinolones such as ciprofloxacin, enoxacin, and norfloxacin. These drug interactions can be easily avoided. Significant decreases in the absorption of fluoroquinolones by metal cations can be prevented by staggering the doses of these drugs. To avoid alterations in methylxanthine metabolism, newer fluoroquinolones, such as lomefloxacin, ofloxacin, and temafloxacin, should be utilized; alternatively, theophylline serum levels can be carefully monitored. Several other potentially serious drug interactions involving cyclosporine, warfarin, and nonsteroidal anti-inflammatory drugs have been reported, but additional investigations are required before their overall clinical significance can be fully determined. Since the use of fluoroquinolones will continue to escalate over the next decade, continued patient surveillance is necessary so that potential drug interactions can be recognized, described, and prevented.
The processivity of the DNA polymerase alpha-primase complex from calf thymus was analyzed under various conditions by Hohn KT, Grosse F. When multi-RNA-primed M13 DNA was used as the substrate, the DNA polymerase alpha-primase complex was found to incorporate 19 +/- 3 nucleotides per primer binding event. This result was confirmed by product analysis on sequencing gels following DNA synthesis on poly(dT) X (rA)10. The processivity depends strongly on the assay conditions but does not correlate with enzymic activity. Lowering the concentration of Mg2+ ions to less than 2 mM increases the processivity to 60. Replacing Mg2+ by 0.2 mM Mn2+ results in 90 nucleotides being incorporated per primer binding event. Neither the presence of ATP nor the addition of noncognate deoxynucleotide triphosphates affects the processivity of the DNA polymerase alpha-primase complex. Lower processivity was induced by lowering the reaction temperature, by adding spermine, spermidine, or putrescine, in the presence of the antibiotics novobiocin and ciprofloxacin, by adding Escherichia coli single-stranded DNA binding protein, or by adding calf thymus topoisomerase II and RNase H. Three single-stranded DNA binding proteins from calf thymus, including unwinding protein 1, do not affect processivity to any significant extent. Freshly prepared DNA polymerase alpha-primase complex exhibits in addition to its processivity of 20 further discrete processivities of about 55, 90, and 105. This result suggest that further subunits of the polymerase alpha-primase complex are necessary to reconstitute the holoenzyme form of the eukaryotic replicase.

The uptake of fleroxacin was reduced and its MIC was increased in the presence of magnesium. Quinolones induced lipopolysaccharide release, increased cell-surface hydrophobicity and outer membrane permeability to B-lactams, and sensitized cells to lysis by detergents. These effects were also antagonized by magnesium and were very similar to those seen with EDTA and gentamicin. MICs of quinolones in portin-deficient strains were increased relative to those of the
parent strain, consistent with a porin pathway of entry. However, MICs were further increased in the presence of magnesium; the size of the additional increase showed a positive correlation with quinolone hydrophobicity in an OmpF- Omp C- OmpA- strain. When quinolones were mixed with divalent cations in solution, changes in quinolone fluorescence suggestive of metal chelation were observed. The addition of fleroxacin to a cell suspension resulted in a rapid initial association of fluorescence with cells, followed by a brief decrease and a final time-dependent linear increase in cell-associated fluorescence. We interpret these results as representing chelation of outer membrane-bound magnesium by fleroxacin and other quinolones, dissociation of the quinolone-magnesium complex from the outer membrane, and diffusion of the quinolone through both porins and exposed lipid domains on the outer membrane. For a given quinolone, the contribution of the porin and nonporin pathways to total uptake is influenced by the hydrophobicity of the quinolone.23

The complexation of iron(III) with norfloxacin in acidic solution at 25 degrees C, at an ionic strength of about 0.3 M and a pH of 3.0 has been studied. The water-soluble complex formed, which exhibits an absorption maximum at 377 nm, was used for the spectrophotometric determination of trace amounts of iron(III). The molar absorptivity was 9.05 x 10(3) I mol-1 cm-1 and the Sandell sensitivity 6.2 ng cm-2 of iron(III) per 0.001 A. The formation constant (Kf) was determined spectrophotometrically and was found to be 4.0 x 10(8) at 25 degrees C. The calibration graph was rectilinear over the range 0.25-12.0 p.p.m. of iron(III) and the regression line equation was A = 0.163c - 0.00042 with a correlation coefficient of 0.9998 (n = 9). Common cations, except cerium (IV), did not interfere with the determination. The results obtained for the determination of iron(III) using the described procedure and the thiocyanate method were compared statistically by means of the Student t-test and no significant difference was found.24
The uptake of the quinolone drug norfloxacin by Escherichia coli was investigated at initial rate kinetics at different pH and monovalent/divalent metal ion concentration. The results support a simple diffusion mechanism for quinolone incorporation into cells. The uptake process decreases under acidic conditions. The presence of Na+ or K+ ions does not affect the results to an appreciable extent, whereas divalent ions cause a dramatic decrease in drug incorporation. The antibacterial activity, evaluated under identical experimental conditions, shows a direct relationship with the uptake data. As a general explanation for the above results it is suggested that the ability of the drug to penetrate into cells is a function of its net charge. The molecule in the zwitterionic form exhibits maximum permeation properties, whereas the uptake is remarkably reduced when the drug bears a net charge as a result of ionization or complex formation with bivalent ions. These results allow further insight into the mechanism of quinolone access to the intracellular compartment.

The effect of ferrous sulphate (300 mg), ferrous gluconate (600 mg), and a combination tablet of iron (10 mg), magnesium (100 mg), zinc (15 mg), calcium (162 mg), copper (2 mg), and manganese (5 mg) (Centrum Forte) co-administration on ciprofloxacin bioavailability was tested in eight healthy subjects. 2. Peak serum ciprofloxacin concentrations and area under the curve (AUC) were significantly reduced when ciprofloxacin was administered with 300 mg ferrous sulphate (3.0 vs 2.0 mg l-1, P less than 0.05 and 12.3 vs 6.7 mg l-1 h, P less than 0.01, respectively). Reductions in peak ciprofloxacin concentrations and AUC also occurred when ciprofloxacin was ingested with 600 mg ferrous gluconate (1.3 mg l-1, P less than 0.01 and 4.1 mg l-1 h, P less than 0.01, respectively) and a Centrum Forte tablet (1.4 mg l-1, P less than 0.01 and 5.4 mg l-1 h, P less than 0.01, respectively). 3. When ferrous ion was mixed with ciprofloxacin, rapid spectral changes occurred (t1/2 = 1.9 min). Additional studies were consistent with
oxidation of the ferrous form of iron to its ferric form, which is followed by rapid formation of a Fe(3+)-ciprofloxacin complex. Ciprofloxacin seems to bind to ferric ion in a ratio of 3:1 by interacting with the 4-keto and 3-carboxyl groups on ciprofloxacin. 4. The formation of a ferric ion-ciprofloxacin complex is probably the cause of the reduction in ciprofloxacin bioavailability in the presence of iron.26

The binding of plasmid DNA to norfloxacin, a quinolone antibacterial agent, was investigated by fluorescence, electrophoretic DNA unwinding, and affinity chromatography techniques. The amount of quinolone bound to DNA was modulated by the concentration of Mg2+. No interaction was evident in the absence of Mg2+ or in the presence of an excess of Mg2+, whereas maximum binding was observed at a Mg2+ concentration of 1-2 mM. The experimental data can be fitted to the formation of three types of Mg adducts: a binary adduct with norfloxacin and Mg2+, a binary adduct with DNA and Mg2+, and a ternary adduct with quinolone, plasmid, and Mg2+. We propose a model for the ternary complex, in which Mg acts as a bridge between the phosphate groups of the nucleic acid and the carbonyl and carboxyl moieties of norfloxacin. Additional stabilization may arise from stacking interactions between the condensed rings of the drug and DNA bases (especially guanine and adenine), which may account for the preference exhibited by quinolones for single-stranded and purine-rich regions of nucleic acids. Other possible biochemical pathways of drug action are suggested by the observation that norfloxacin binds Mg2+ under conditions that are close to physiological.27

The primary target of fluoroquinolones has been identified as the enzyme DNA gyrase, but the mechanism of action of these antibacterial agents is still a matter of controversy. Using partitioning in aqueous polyethylene glycol (PEG)-dextran systems, the affinities of several fluoroquinolones for DNA were determined with accuracy and at equilibrium. It was proved that the binding was
strongly dependent on the ability of the drugs to bind Mg$^{2+}$, with KA values of about 40 000 l mol$^{-1}$, but was poorly related to the antibacterial activity [minimal inhibitory concentration (MIC) and gyrase inhibition]. Using affinity chromatography on immobilized fluoroquinolone, it was shown that DNA gyrase was unable to bind fluoroquinolones in the absence of DNA, but that a DNA-quinolone-gyrase complex was formed in the presence of Mg$^{2+}$.$^{28}$

The interaction between divalent cations and quinolones and the mechanism by which the former antagonizes the antimicrobial activities of the latter were investigated. In the presence of either magnesium or calcium chloride, the MICs of 18 quinolones for Gram-positive and Gram-negative bacteria increased. Accumulation of and inhibition of DNA synthesis by quinolones were decreased in the presence of magnesium chloride while, in the presence of EDTA, there was no increase in the concentration of accumulated quinolone for any of the agents tested. Only with nalidixic acid was there enhancement of the inhibition of DNA synthesis. Chelation of selected quinolones by magnesium was demonstrated with a fluorescence assay which showed that the extent to which fluorescence (consistent with chelation) was enhanced varied with the quinolone. Assessment of the strength of the magnesium-quinolone complexes with the chelating agent EDTA demonstrated that some of the complexes could be broken. Thin layer chromatography of quinolones and quinolone-magnesium complexes provided evidence that the components of the complex were probably combined in a ratio of 1:1 and that reduced intracellular accumulation of the quinolones in the presence of magnesium was unlikely to be due to a complex being too bulky to be taken through the porin channels. In contrast with permeabilizers which are known to utilize the self-promoted uptake pathway, none of the quinolones studied permeabilized Gram-negative bacteria to lysozyme, caused enhanced fluorescence to 1-N-phenyl 1-naphthylamine (NPN) or increased the leakage of periplasmic beta-lactamase into
the culture medium. The reduced activities of the quinolones in the presence of
divalent cations may be the result of the chelation of exogenous ions and, possibly,
lipopolysaccharide- or lipoteichoic acid-associated magnesium ions, thereby
resulting in less drug being available to enter the bacterium. Alternatively, reduced
activity may be due to a fundamental effect on the interaction between quinolones
and their target DNA gyrase.\textsuperscript{29}

The effect of zinc supplementation on intestinal permeability changes
and protein loss was studied in 32 children aged between 1 and 12 years during
bouts of acute shigellosis and after recovery. An intestinal permeability test and
then a 48 hour balance study were performed on all patients. They were then blindly
assigned to receive vitamin B syrup either with or without zinc acetate (15 mg/kg
per day) for a month. All patients received a five day course of nalidixic acid. The
balance study was repeated during convalescence and follow up, but a permeability
test was done only at follow up after one month. Intestinal permeability, expressed
as a urinary lactulose:mannitol excretion ratio, improved significantly (p = 0.001)
along with a significant increase (p = 0.005) in mannitol excretion in the zinc
supplemented children, suggesting a resolution of small bowel mucosal damage.
The latter was associated with a higher coefficient of nitrogen absorption (p = 0.03),
suggesting a possible role of zinc in the treatment of shigellosis. Enteric protein
loss, as assessed by faecal alpha 1 antitrypsin clearance, was not influenced by zinc
supplementation.\textsuperscript{30}

Simultaneous administration of antacids containing magnesium or
aluminium and ciprofloxacin or other quinolones decreases the gastrointestinal
absorption of those antibacterial agents. Current speculation about the mechanism
of this interaction has focused on drug-cation chelation. The present study was
designed to detect the protonation in solutions and the formation of the complex
species at the pH levels typical of the gastrointestinal tract. It involves the study of
ciprofloxacin in aqueous solutions containing Al3+ and (or) Mg2+ by combining the results of potentiometric and spectroscopic (1H nuclear magnetic resonance) techniques. Calculations were only performed for data in the range $4.5 < \text{pH} < 5.5$ (pH levels typical of gastrointestinal tract) and the results of both methods are made self-consistent, assuming an equilibrium model including complex species MHL, MLOH (where H2L denotes ciprofloxacin and M is Al3+ or Mg2+).\textsuperscript{31}

Three novel complexes of norfloxacin (abbreviated as NFL), [M(NFL)2(H2O)2]Cl3.6H2O, (M = Fe, Co), and [Zn(NFL)2]Cl2.7H2O, have been prepared. The compounds were characterized by IR, UV-Vis, NMR spectra, molar conductivity, and elemental analyses.\textsuperscript{12}

The quinobenzoxazine compounds A-62176 and A-85226 belong to a novel class of antineoplastic agents that are catalytic inhibitors of topoisomerase II and also structural analogs of the antibacterial DNA gyrase inhibitor Norfloxacin. In vitro studies have shown that their antineoplastic activity is dependent upon the presence of divalent metal ions such as Mg2+ and Mn2+, although the precise role of these ions in the mechanism of action is unknown. In this study we have investigated the structures of the binary complex between the quinobenzoxazines and Mg2+ and the ternary complex between quinobenzoxazine-Mg2+ and DNA. The stoichiometry of the binary and ternary complexes and the biophysical studies suggest that a 2:2 drug:Mg2+ complex forms a "heterodimer complex" with respect to DNA in which one drug molecule is intercalated into DNA and the second drug molecule is externally bound, held to the first molecule by two Mg2+ bridges, which themselves are chelated to phosphates on DNA. There is a cooperativity in binding of the quinobenzoxazines to DNA, and a 4:4 drug:Mg2+ complex is proposed in which the two externally bound molecules from two different 2:2 dimers interact via pi-pi interactions. The externally bound quinobenzoxazine molecules can be replaced by the quinolone antibacterial compound Norfloxacin to form mixed-
structure dimers on DNA. Based upon the proposed model for the 2:2 quinobenzoxazine:Mg2+ complex on DNA, a parallel model for the antibacterial quinolone-Mg2(+)−DNA gyrase complex is proposed that relies upon the ATP-fueled unwinding of DNA by gyrase downstream of the cleavable complex site. These models, which have analogies to leucine zippers, represent a new paradigm for the structure of drug-DNA complexes. In addition, these models have important implications for the design of new gyrase and topoisomerase II inhibitors, in that optimization for structure-activity relationships should be carried out on two different quinolone molecules rather than a single molecule.33

Reaction of the fluoroquinolone antimicrobial ciprofloxacin with copper(II) nitrate in the presence of 2,2'−bipyridine resulted in the isolation of the complex [Cu(cip)(bipy) (Cl)0.7(NO3)0.3] (NO3).2H2O. Reaction of an aqueous solution of ciprofloxacin.HCl and NaCl with CuCl2 at pH 5.0 resulted in the isolation of [Cu(cip)2]Cl2.11H2O. The complex [Cu(cip) (bipy)(Cl)0.7(NO3)0.3] (NO3).2H2O crystallizes in the monoclinic space group P2(1)/n, with a = 13.955(8), b = 14.280(8), c = 14.192(6) Å, beta = 93.10(4) degrees, Z = 4 with R = 0.046. The selective broadening of resonances in the 13C NMR spectrum of ciprofloxacin by the addition of Cu2+(aq) was employed to probe metal ion binding sites in the ligand. The protonation constants of norfloxacin and ciprofloxacin, and the formation constants with copper(II), were determined by potentiometric titrations at 25 degrees C. The additions of ciprofloxacin to metal to form ML and ML2 complexes exhibit stepwise formation constants of log K1 6.2(1) and log K2 11.1(3), respectively.34

Spectrophotometric and spectrofluorimetric methods for the determination of two broad-spectrum fluoroquinolone antibacterials (ciprofloxacin and norfloxacin), either in pure form or in tablets, are described. Both methods are based on the formation of a ternary complex between palladium(II), eosin and the
fluoroquinolone in the presence of methyl cellulose, as surfactant. Spectrophotometrically, under the optimum conditions, the ternary complexes showed an absorption maximum at 545 nm, with apparent molar absorptivities of 3.4 x 10^4 and 2.7 x 10^4 mol-l cm^-1 and Sandell's sensitivities of 1.01 x 10^-2 and 1.12 x 10^-2 micrograms cm^-2 for ciprofloxacin and norfloxacin, respectively. The solution of the ternary complex obeyed Beer's law in the concentration range 3-10 micrograms ml^-1 for both quinolones. The proposed method was applied to the determination of the two drugs in pharmaceutical tablets. A fluorescence quenching method for the determination of both quinolones by forming this ternary complex was also investigated for the purpose of enhancing the sensitivity of the determination. The results obtained by the application of both procedures and the USP XXIII methods were in good agreement and statistical comparison by means of Student's t-test and the variance ratio F-test showed no significant differences between the three methods.35

The formation constants of the fluoroquinolones norfloxacin and ciprofloxacin with Mg2+ (log beta 1 = 2.97(4), log beta 2 = 5.6(2)), Zn2+ (log beta 1 = 3.77(2), log beta 2 = 7.59(3)), and Fe2+ (log beta 1 = 3.99(5), log beta 2 = 7.2(5)) were determined by potentiometric titration. The pH at which precipitation occurred in the titration solutions was compared for the metal ions Ca2+, Mg2+, Zn2+, Fe2+, Cu2+, and Al3+. The formation constants were used to predict a rank order of metals that may be expected to hinder the gastrointestinal absorption of the fluoroquinolones, in vivo. The effects of metal ions on the pharmacokinetics of orally-administered norfloxacin in the dog were investigated. Norfloxacin (12 mg/kg) was administered alone or with equimolar doses of each of the chloride salts of Ca2+, Mg2+, Zn2+, Fe2+, and Al3+. Statistically significant reductions in serum norfloxacin concentrations were observed after analysis by HPLC. The Cmax was reduced 29-85%, while the area under the norfloxacin serum concentration-time
curve (AUC0-infinity) was reduced by 29-79%. The extent of the reduction in AUC0-infinity was correlated with the magnitude of the formation constant of the 1:1 norfloxacin:metal chelate complex for the divalent metal ions. On coadministration of 12 mg/kg norfloxacin with various doses of Mg2+ (chloride) the AUC0-infinity and Cmax decreased with increasing Mg2+ dose. The interaction peaked at a Mg2+:norfloxacin ratio of 1:2 suggesting the formation of a 1:2 Mg:norfloxacin complex. Formation constant data were used to simulate the percentage of norfloxacin complexed at pH 6.5. Combinations of metal ion and norfloxacin which result in only a small extent (< 20%) of norfloxacin complex formation can result in relatively large decreases in oral bioavailability of this antimicrobial agent.36

Studies of complexation equilibria of the antibiotic anions nalidixate and cinoxacinate with [Cu(phen)]2+ and [Cu(bipy)]2+ are reported. These studies indicate that the stability of this type of complex is strongly related to the metal environment. A correlation between the stability constants, determined here, with the sigma donation character of the ligand is proposed. This study shows that the stability constant for the reaction between the quinolones and the moiety [Cu(N-N)]2+ is dependent on the coordinate diamine to the metal ion. This is in agreement with previous studies where other physical properties as their electronic absorption spectra in the visible region, display similar behavior. These results suggest that inside the living cells, a possible interaction with some metal ion will be strongly controlled by the type of ligand bound to the cation.37

Norfloxacin (NOR)(1a) forms an inclusion complexe with betacyclodextrin (BCD) (1c) by neutralisation method. The carboxyl function of norfloxacin is found to interact with the hydroxyl groups of betacyclodextrin present at the open end through hydrogen bonding as evidenced by spectral analysis.38
Ciprofloxacin (CIP) forms an inclusion complex with betacyclodextrin. The dissolution of the complex in a buffer solution of pH 1.2 and 7.2 and the diffusion at pH 1.2 was better than the plain drug. The stability of the drug under accelerated stability conditions was better on complexation. The in vitro and in vivo release of the complex in the rabbit ophthalmic cavity were correlated and compared with the plain drug.\(^{39}\)

A simple, rapid and sensitive spectrophotometric method is developed for the determination of norfloxacin in bulk drugs and in pharmaceutical preparations. The method is based on chloroform extraction of yellow colour product obtained by ceric (IV) oxidation in acidic medium. The colour is stable for about 24 hours after its extraction. Beer's law is obeyed over the range of 10-100 mcg/ml. The method has been applied successfully for the analysis of norfloxacin in tables and eye-drops.\(^{40}\)

Norfloxacin (NOR) is complexed with betacyclodextrin (BCD) by neutralisation method. The dissolution and diffusion in buffer solution of different pH and the stability at accelerated stability conditions of the complex were found better than that of the plain drug or the physical mixture. The complex and the plain drug were incorporated into polymeric matrices and the implants thus prepared were used as ophthalmic implants in rabbits. The in vitro and in vivo release of the complex was found better than the plain drug. Thus the complex can be utilised more effectively than the plain drug as ocular implants for local drug delivery.\(^{41}\)

Simple, rapid, accurate and sensitive spectrofluorimetric methods for the determination of norfloxacin are described. The methods are based on the reaction of this drug with aluminium(III) ion to form a strongly fluorescent complex. Fluorescence properties of the AlIII-norfloxacin complex were used for the determination of this drug in pharmaceutical preparations. First-derivative
constant wavelength synchronous fluorescence spectrometry was used for the determination of norfloxacin in the presence of nalidixic acid. The determination of norfloxacin in urine without the need of tedious pre-separation was achieved by using zero-crossing second-derivative synchronous fluorescence spectrometry.\textsuperscript{42}

Interactions between ciprofloxacin (CPFX), Mg\textsuperscript{2+}, Mn\textsuperscript{2+} and DNA, and their polarographic and voltammetric behaviour were studied. In 0.1 mol.L\textsuperscript{-1} NH\textsubscript{3}-NH\textsubscript{4}Cl buffer solution (pH 9.2), a new reduction peak was obtained by linear-sweep voltammetry with Ep = -1.72 V(vs Ag/AgCl) when adding DNA to CPFX solution, which implies binding of CPFX with DNA. In the presence of Mg\textsuperscript{2+} or Mn\textsuperscript{2+}, another new sensitive reduction peak, whose peak potential is more negative (Ep = -1.78 V), was obtained which suggested that Mg\textsuperscript{2+} or Mn\textsuperscript{2+} took part in the interaction between CPFX and DNA resulting in a ternary complex of CPFX-Mg-DNA. The peak current (ip) is proportional to the concentration of DNA over the range of 1.18 x 10\textsuperscript{-4}-3.33 x 10\textsuperscript{-4} mol.L\textsuperscript{-1}. In this paper, the properties of the peak current were studied in detail, the result showed that the electrode reaction was irreversible and the ip was influenced by adsorption. The electrode reaction mechanism was also probed into. The CPFX molecule in the complex was reduced on the electrode.\textsuperscript{43}

It has been proposed that the quinobenzoxazines form a 2:2 drug-Mg(2+) self-assembly complex on DNA. The quinobenzoxazine (S)-A-62176 is photochemically unstable and undergoes a DNA-accelerated photochemical reaction to afford a highly fluorescent photoproduct.\textsuperscript{44}

Gibbs energy, enthalpy and entropy for the reactions of ciprofloxacin and lomefloxacin with aluminium ion were determined. No significant differences were observed for Gibbs energy values for these fluorquinolones while significant differences were found for enthalpy and entropy. The highly positive values
observed for the entropy give evidence for a strong cation-ligand interaction and point out the role of hydration in the complex formation. As the value of the entropy for the reaction with ciprofloxacin is higher than that observed for lomefloxacin one concludes that the former ligand interacts more strongly with the metal ion than the latter.\textsuperscript{45}

The results from the measurement of the fluorescence spectra of fluoroquinolone antibiotics including ofloxacin (OF), norfloxacin (NOR) and ciprofloxacin (CIP) complexed with cobalt (II) and ATP give information concerning the antibiotics-nucleotide interactions. From the fluorescence spectral data, it appears that the fluoroquinolone antibiotic cannot directly complex with ATP but indirectly complex with cobalt (II), which is playing an intermediary role. The interaction of fluoroquinolone antibiotic with the nucleotide occurs mainly through the phosphate group. The conclusion offers a more complete mechanism, which is important for understanding the interaction of these drugs with DNA.\textsuperscript{46}

2D molecular square grid with strong blue fluorescent emission was studied for a complex of norfloxacin with zinc(II).\textsuperscript{47}

Microcin B17 is a 3.1-kDa bactericidal peptide; the putative target of this antibiotic is DNA gyrase. Microcin B17 has no detectable effect on gyrase-catalysed DNA supercoiling or relaxation activities in vitro and is unable to stabilise DNA cleavage in the absence of nucleotides. However, in the presence of ATP, or the non-hydrolysable analogue 5'-adenyllyl beta, gamma-imidodiphosphate, microcin B17 stabilises a gyrase-dependent DNA cleavage complex in a manner reminiscent of quinolones, Ca(2+), or the bacterial toxin CcdB. The pattern of DNA cleavage produced by gyrase in the presence of microcin B17 is different from that produced by quinolones and more closely resembles Ca(2+)-mediated cleavage. Several gyrase mutants, including well-known quinolone-resistant mutants, are cross resistant to microcin-induced DNA cleavage.\textsuperscript{48}
Quinolones and magnesium deficiency cause similar lesions in joint cartilage of young animals. Chondrocytes cultivated in the presence of quinolones and in Mg-free medium show severe alterations in cytoskeleton and decreased ability to adhere to the culture dish. We investigated whether Mg2+supplementation can prevent quinolone-mediated effects on chondrocytes in vitro. Chondrocytes cultivated in Dulbecco's modified Eagle's medium/HAM's F-12 medium were treated with ciprofloxacin (80 and 160 microg/ml) and enrofloxacin (100 and 150 microg/ml). Mg2+ was added at a concentration of 0.0612 mg/ml (MgCl) and 0.0488 mg/ml (MgSO4) or a triple dose. In addition, cells were cultivated in Mg-free medium and accordingly treated with Mg2+ supplementation. After 5 days in culture, the number of adherent cells per milliliter was determined. The number of chondrocytes in quinolone-treated groups decreased to 12-36% that of the control group within the culture period. With Mg2+ supplementation, the number of attached cells increased to 40-70% that of control cells. The threefold dose of Mg2+ led to better results than did the single dose. Cell proliferation tested by immunohistochemical staining with Ki67 (clone MIB5) decreased from 70% in control groups to 55%, 48%, and 30% in enrofloxacin-treated groups in a concentration dependent manner (50, 100, and 150 microg/ml). Addition of Mg2+ did not increase the rate of cell proliferation. These results suggest that a great part of quinolone-induced damage is due to magnesium complex formation, as Mg2+ supplementation is able to reduce the effects in vitro. However, quinolone effects on cell proliferation seem to be an independent process that is not influenced by magnesium supplementation.49

Penicillin G has been found to chelate Cu2+ although the chelating power of the corresponding acylated amino acids is very small50. Under mildly acid condition penicillin G and V have been shown to be catalytically hydrolysed by cupric ion in to corresponding penicillioic acids at rates too much rapid for possible
intact penicillin cupric ion interactions to be measured by standard complexation techniques.\textsuperscript{51-52} The reaction mechanism and the catalytic site of complexation of Cu(II) with penicillin have been studied.\textsuperscript{53}

Kinetic analysis of complex formation between penicillin and sucrose studied by Hem SL, Russo EJ, Bahal SM, Levi RS.\textsuperscript{54}

A sensitive spectrophotometric method is developed for the determination of some antibiotic drugs such as ampicillin (amp), diclaxacillin (dicl), flucloxacinil (fluc) and amoxicillin (amox). The method involves the formation of ion-pairs between these drugs under investigation and inorganic complex of Mo (V) thiocyanate followed by its extraction with methylene chloride. The optimum conditions for the ion-pairs formation are established. The method permits the determination of amp, dicl, fluc and amox over a concentration range of 1.5-77.5, 3-75, 1.5-79 and 7.5-75 microg ml\textsuperscript{-1} respectively. The sensitivity (S) is found to be 0.017, 0.061, 0.014 and 0.073 microg cm\textsuperscript{-2} for amp, dicl, fluc and amox, respectively. The method is simple, rapid, reproducible and accurate within +/- 1%. The method is applicable for the assay of the four drugs under investigation in different dosage forms and the results are in good agreement with those obtained by the official method.\textsuperscript{55}
REFERENCES


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