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The interactions of metal ions with quinolone antibacterial agents

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Contents

Abbreviations: bipy, 2,2′-bipyridine; CD, cyclodextrin; cfH, ciprofloxacin; cxH, cinoxacin; DNA, deoxyribonucleic acid; DMSO, dimethylsulfoxide; DTA, differential thermal analysis; DTG, derivative thermogravimetry; DSC, dynamic scanning calorimetry; ESR, electron spin resonance; EGA, evolved gas analysis; erxH, enrofloxacin; FClH, quinolone without a trivial name (see Scheme 2); GMP, guanosine-5′-monophosphate; kinoH, quinolone without a trivial name (see Scheme 2); M, metal; MIC, minimal inhibitory concentration; nalH, nalidixic acid; nfH, norfloxacin; nta, nitrilotriacetate; ofloH, ofloxacin; oxoH, oxolinic acid; pfH, pefloxacin; phen, 1,10-phenantroline; PPh_3, triphenylphosphine; QH, neutral quinolone molecule; QH_2^+, protonated quinolone molecule; QH_3^{2+}, doubly protonated quinolone molecule; $QH^{\pm}$, zwitterionic form; Q^-, deprotonated quinolone molecule; SAR, structure–activity relationship; TG, thermogravimetry.

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Abstract

The quinolones are a group of synthetic antibacterial agents structurally related to nalidixic acid. The absorption of quinolone drugs is lowered when they are consumed simultaneously with magnesium or aluminium antacids. Many other ions found in pharmaceuticals cause similar effect. The proposed reason for such behaviour is the chelate bonding of the quinolone to the metal. In the review article, selected crystal structures of quinolone–metal compounds are presented and discussed. The results of different physico-chemical methods (thermal analysis, potentiometric measurements, IR, UV–vis, NMR spectroscopy) as well as some results of bioactivity tests are also included.

Keywords: Quinolones; Metal ions; Crystal structures; Spectroscopy; DNA; Bioactivity

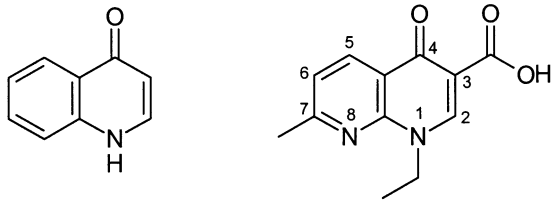
1. Introduction

1.1. General aspects and characteristics of quinolones

The term quinolones is commonly used for the quinolonecarboxylic acids or 4-quinolones, which are a group of synthetic antibacterial agents containing a 4-oxo-1, 4-dihydroquinoline skeleton (Scheme 1). Since the introduction of nalidixic acid (nalH) [1] (Scheme 1) into clinical practice in the early 1960s, a number of structurally related highly potent broad-spectrum antibacterial agents has been isolated [1,2].

Modifications of nalH were made based on structure–activity-relationships (SARs). It was discovered that a fluorine atom at position 6 and a piperazine ring at position 7 greatly enhance the spectrum of activity. The fluoroquinolones are very active against aerobic Gram-negative microorganisms but less active against Gram-positive microorganisms [1,3]. They are extremely useful for the treatment of a variety of infections, including urinary tract infections, soft tissue infections, respiratory infections, bone-joint infections, typhoid fever, sexually transmitted diseases, prostatitis, community-acquired pneumonia, acute bronchitis and sinusitis [1,2,4]. Recently, a relatively new approach to the rational design of antitumor agents has been introduced based on some new quinolone molecules that display a novel mode of action [5].

The formulas of the quinolones mentioned in this review are presented in Scheme 2 but many other quinolones are known and are also used in clinical practice nowadays.



Scheme 1. The formulas of 4-oxo-1, 4-dihydroquinoline (left) and nalidixic acid (right).

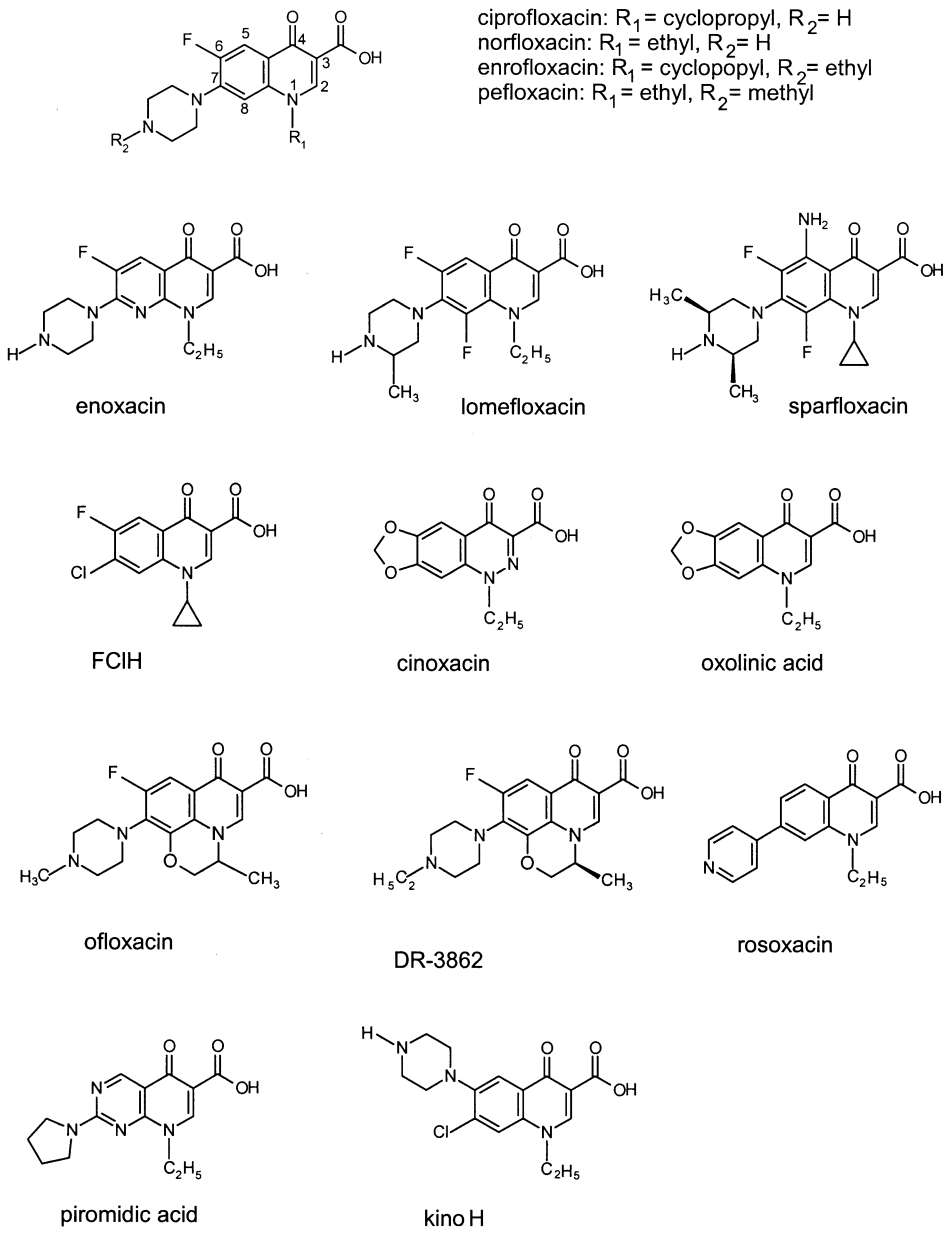
1.2. The influence of metal ions on the activity of quinolones

Höffken et al. [6] first reported that concurrent administration of magnesium–aluminium containing antacid with ciprofloxacin (cfH) resulted in a nearly complete loss of activity of the drug in serum.

Antacids not only contain magnesium or aluminium, but can also contain calcium or bismuth ions. In the treatment of anaemia, iron is orally administered while zinc is present in multivitamin mixtures. Several authors have begun to study the reasons for the reduced activity of quinolones in the presence of the ions mentioned above [7–23]. According to these results, it was suggested that multivalent cations should be avoided in patients receiving quinolone antibacterials.

The uptake of norfloxacin (nfH) by *Escherichia coli* was investigated at different pH and monovalent–divalent metal ion concentrations [24]. The results of the study supported a simple diffusion mechanism for quinolone incorporation into cells. The uptake process decreases under acidic conditions. The presence of Na^+ and 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. It was suggested that the ability of the drug to penetrate into cells is a function of its net charge. The molecule in zwitterionic form exhibits maximum permeation properties, whereas the uptake is strongly reduced when the drug bears a net charge as a result of ionization or complex formation with divalent ions.

The proposed mechanism of the interaction between quinolone and metal cations was chelation between the metal and the 4-oxo and adjacent carboxyl groups. Since these functional groups are required for antibacterial activity, it could be anticipated that all of the quinolones will interact with metal ions. However, the authors proposed that there may be differences between the quinolones regarding the extent of interaction [7].



Scheme 2. The formulas of quinolone molecules that appear in the text.

2. Crystal structures of quinolones and their metal compounds

2.1. Free drugs

The crystal structures of several free quinolone molecules have been determined: nalidixic acid (nalH) [25,26], pefloxacin (pfH) methanesulfonate [27,28], cinoxacin (cxH) [29], nfH [30,31], cfH hexahydrate [32], cfH lactate [33], norfloxacin dihydrochloride [34,35], 5-aminooxolinic acid [36], oxolinic acid [37], lomefloxacin [38], rosoxacin [39], piromidic acid [40,41] and sparfloxacin [42]. It is interesting to note that in most cases the carboxylic group is not deprotonated and the hydrogen atom of this group is hydrogen bonded to an adjacent 4-oxo atom. In a few examples [30–32,42], the carboxylic group is ionized and the molecule thus exists in a zwitterionic form with protonated terminal nitrogen of the piperazine ring in a solid state.

2.2. Metal and boron complexes

All quinolones are sparingly soluble in water in the pH range between 4 and 9. Mixing of a water solution of metal salt and a quinolone solution mostly results in a precipitation, making it difficult to grow crystals of complexes. Nevertheless, the complexes of silver, cobalt, zinc, cadmium, boron and a few copper complexes have

been prepared and their crystal structures reported in the literature. In addition some mixed ligand complexes have been isolated. A real breakthrough in the isolation of quinolone–metal complexes was achieved by using hydrothermal reactions introduced by the group of Xiao-Zeng You [43–45]. They have prepared complexes with quinolones coordinated to magnesium, calcium, copper(I) and zinc. In some of these crystal structures the bonding modes are significantly different from those reported before. All of these structures are discussed below. The numberings from original papers have been used throughout the text.

2.2.1. Magnesium complex

A complex of magnesium(II) with the formula $[Mg_2(H_2O)_6(nfH)_2]Cl_4\cdot 4H_2O$ was isolated by the hydrothermal reaction [43]. It can be described as a 2:2 dimer in which the two magnesium ions are bridged by two oxygen atoms from carboxylate groups of the two norfloxacin molecules. Each magnesium ion is octahedrally coordinated with the oxygen atom of the quinolone carbonyl (Mg(1)–O(1) = 1.997(2) Å), one of the two oxygen atoms of the carboxylate (Mg(1)–O(2) = 2.084(2), Mg(1)–O(2A) = 2.116(2) Å) and water molecules. The coordination mode of carboxylate can be considered as a monodentate bridging type.

2.2.2. Calcium complex

A calcium complex, $[Ca_2(Cl)(nfH)_6]Cl_3\cdot 10H_2O$ was also isolated by the hydrothermal reaction [43]. This complex is also a dimer, but the bridging group is a chloride ion. The coordination geometry around each calcium ion can best be described as a distorted pentagonal bipyramid. Three norfloxacin molecules act in a bidentate coordination mode through the oxygen atom of the quinolone carbonyl (Ca(1)–O = 2.384(2)–2.413(2) Å) and one of the two oxygen atoms in the carboxylate moiety (Ca(1)–O = 2.383(3)–2.395(3) Å). The chloride ion completes the seven-coordination around the calcium (Ca(1)–Cl(1) = 2.862(9) Å).

2.2.3. Boron complexes

Boron complexes have been used in the synthesis of some quinolone molecules. The crystal structures of two boron complexes have been reported. In the difluoroboric–quinolone complex [46], boron is coordinated to ring carbonyl oxygen (B–O(4) = 1.468 Å) and to the oxygen atom of a carboxylic group (B–O = 1.467 Å).

In the bis(acetato)-quinolone complex [47] the boron atom is coordinated by four oxygen atoms and adopts a slightly distorted tetrahedral geometry. The boron atom is bonded to the carboxylic (B(1)–O(1) = 1.501(4) Å) and carbonyl (B(1)–O(3) = 1.458(5) Å) atoms of a quinolone molecule. The coordination sphere is completed by two oxygen atoms of the acetate groups (Fig. 1).

2.2.4. Vanadium complex

A vanadium complex [48] was prepared from a water solution of $VOSO_4$ and cfH. The crystals were very unstable and contained a high amount of disordered water molecules, so the exact solution of the structure has not yet been possible. The tentative formula of the complex is $[VO(cfH)_2]SO_4\cdot 10H_2O$ with a typical chelate bonding of metal to 4-oxo and carboxylic oxygens of quinolone.

2.2.5. Iron complex

The iron(III) complex with cfH and nitriloacetate (nta) as ligands, $[Fe(cfH)(nta)]\cdot 3.5H_2O$ was isolated from water solutions of cfH·HCl, $Fe(NO_3)_3$ and nitrilo-

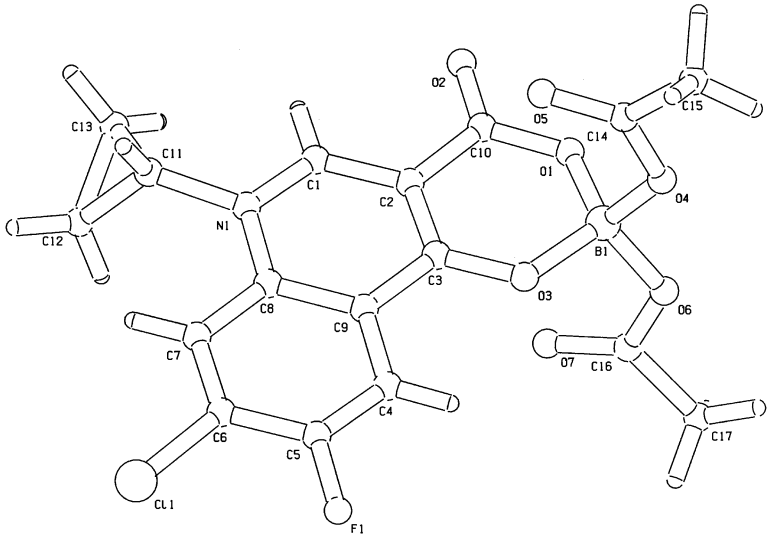


Fig. 1. View of the boron–quinolone complex. Adapted from Ref. [47].

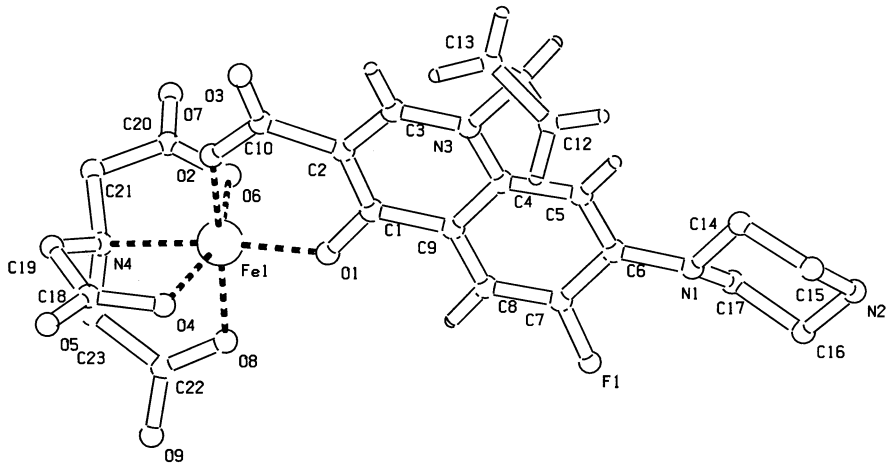


Fig. 2. View of [Fe(cfH)(nta)] complex. Adapted from Ref. [49].

triacetate (nta) (disodium salt) [49]. Dilute ammonia solution was used to adjust the pH to 7. The structure consists of a neutral [Fe(cfH)(nta)] complex and 3.5 water molecules (Fig. 2). The iron is coordinated to the keto (Fe(1)–O(1) = 1.942(8) Å) and the carboxylic oxygen (Fe(1)–O(2) = 1.91(1) Å) of the cfH ligand to form a six-membered ring. The remaining four coordination sites are occupied by an nta ligand (Fe(1)–O(4) = 2.037(9) Å, Fe(1)–O(6) = 1.969(9) Å, Fe(1)–O(8) = 1.983(9) Å, Fe(1)–N(4) = 2.21(1) Å). The piperazinyl ring of the cfH ligand is protonated on the external nitrogen.

2.2.6. Cobalt complex

A compound of cobalt(II) with the formula $Na[Co(cx)_3]\cdot 6H_2O$ was isolated from a methanolic solution of cxH and $CoSO_4$ [50] (Fig. 3). The structure consists of anionic monomeric units of $[Co(cx)_3]^-$ and uncoordinated water molecules that provide crystalline stability through a network of hydrogen bond interactions. Three cinoxacinate ions are chelated through oxo (Co(1)–O(3) = 2.166(9) Å) and carboxylic (Co(1)–O(1) = 2.182(8) Å) oxygens.

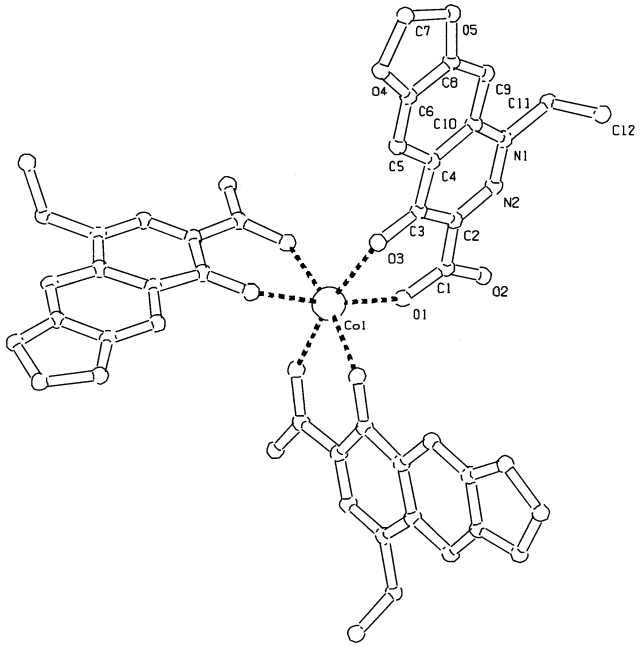


Fig. 3. Perspective view of $[Co(cx)_3]^-$ anion. Adapted from Ref. [50].

2.2.7. Nickel complexes

A mixed ligand complex $[Ni(cx)_2(DMSO)_2]\cdot 4H_2O$ was prepared from a dimethylsulfoxide (DMSO) solution of cxH and $NiSO_4$ [51].The structure of the complex consists of monomeric $[Ni(cx)_2(DMSO)_2]$ units and uncoordinated water molecules that provide crystalline stability through a network of hydrogen bond interactions. The metal ion is bonded to two bidentate cinoxacinate ligands that bind through one carboxylate oxygen atom (Ni–O(1) = 2.003(2) Å) and the exocyclic carbonyl oxygen atom (Ni–O(3) = 2.015(2) Å). The octahedral coordination environment is completed by two DMSO molecules coordinated via the oxygen atom (Ni–O(6) = 2.140(2) Å).

Later on, the same authors reported the complex with the same formula but a slightly different crystal structure [52].

2.2.8. Copper complexes

Two copper(II) complexes were prepared with cfH, two with cxH and one with ofloxacin. Additionally, five mixed ligand complexes were also isolated.

Both copper–cfH complexes were prepared from water solutions of cfH and copper(II) salts (chloride, sulphate). In the complex $[Cu(cfH)_2Cl_2]\cdot 6H_2O$ the copper atom is positioned at the center of inversion and surrounded by four oxygen atoms, Cu(1)–O(1) = 1.928(2) Å and Cu(1)–O(2) = 1.931(2) Å [53] (Fig. 4). The two chloride ions are axially coordinated to copper at longer distances (2.688(2) Å) and appear to be

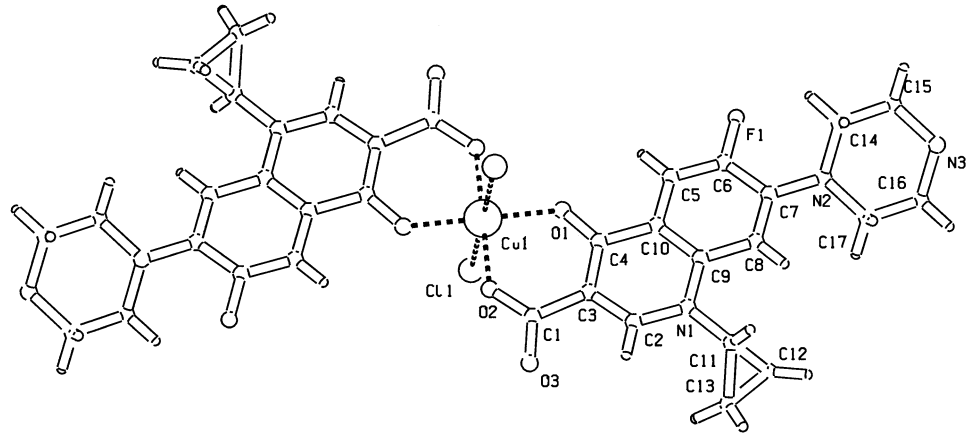


Fig. 4. Perspective view of $[Cu(cfH)_2Cl_2]$. Adapted from Ref. [53].

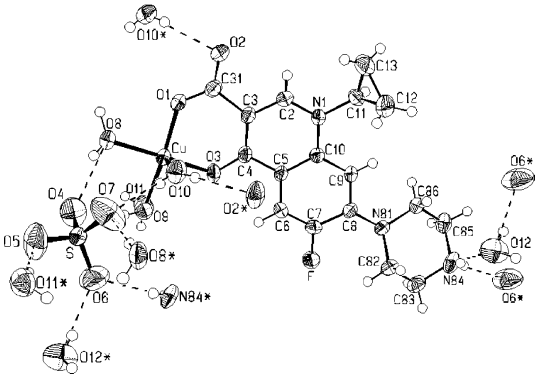


Fig. 5. View of $[Cu(cfH)(H_2O)_3]SO_4 \cdot 2H_2O$. Reproduced with permission—Ref. [54].

disordered over three positions. In the second complex of copper and cfH $[Cu(cfH)(H_2O)_3]SO_4 \cdot 2H_2O$ [54] (Fig. 5), only one molecule of quinolone is coordinated to the metal. The coordination environment around the central copper(II) ion in the structure is a slightly distorted square pyramid. Ciprofloxacin is bonded to the metal through a carbonyl atom (Cu–O(3) = 1.939(1) Å) and a carboxylic atom (Cu–O(1) = 1.915(2) Å). Two water molecules are coordinated to copper in the basal plane (Cu–O(8) = 1.972(2) Å) and (Cu–O(9) = 1.989(2) Å). The apical water molecule is coordinated at a longer distance (Cu–O(10) = 2.174(2) Å). The formulas of the copper complexes of cxH are $[Cu(cx)_2] \cdot 2H_2O$ and $[Cu(cx)_2(H_2O)] \cdot 3H_2O$ [55,52].

In the former, the copper(II) ion is coordinated to two cinoxacinate anions through a carbonyl oxygen atom (Cu(1)–O(3) = 1.903(4) Å) and carboxylate oxygen (Cu(1)–O(1) = 1.896(4) Å), thus forming a CuO_4 chromophore in a crystallographically planar configuration (Fig. 6). The complex was isolated from a DMSO solution of cxH and $Cu(NO_3)_2$.

The latter compound was isolated from the first compound, which was dissolved in aqueous ammonia. The copper(II) ion is found in a distorted square pyramidal environment in the second complex. It is coordinated to four oxygen atoms of the two cinoxacinate ligands (Cu–O(3) = 1.943(5) Å, Cu–O(1) = 1.933(4) Å) and a water molecule (Cu–O(1W) = 2.226(5) Å).

The copper(II) complex with ofloxacin, $[Cu(oflo)_2(H_2O)] \cdot 2H_2O$ was isolated [56]. The geometry around copper(II) ion is a slightly distorted square base pyramid with ordinary chelate bonding of quinolone to the metal through ring carbonyl (Cu(1)–O(33) = 1.956(3), Cu(1)–O(13) = 1.943(3) Å) and one of the carboxylic oxygen atoms (Cu(1)–O(11) = 1.911(3), Cu(1)–O(31) = 1.922(3) Å). Additionally, a water molecule is coordinated to the metal (Cu(1)–O(1) = 2.198(7) Å).

In the complex $[Cu(phen)(nal)(H_2O)]NO_3 \cdot 3H_2O$ [57] (Fig. 7) the copper ion displays a distorted square-pyramidal coordination, being linked to two nitrogen atoms of the 1,10-phenantroline (phen) ligand (Cu–N(11) = 1.999(2) Å, Cu–N(22) = 2.014(2) Å), two oxygen atoms of the nal ligand (Cu–O(11) = 1.914(2) Å, Cu–O(2) = 1.934(2) Å) and a water molecule in the apical site (Ow(1)–Cu = 2.277(3) Å).

A distorted square-pyramidal coordination around copper(II) was also found in the $[Cu(phen)(cx)(H_2O)]NO_3$ complex [58] with similar bonding to that described above. The bond distances to the metal are: (Cu(1)–N(15) = 1.995(5) Å, Cu(1)–N(24) = 2.002(7) Å, Cu(1)–O(15) = 1.913(6) Å, Cu(1)–O(2) = 1.914(4) Å, Cu(1)–O(1W) = 2.238(7) Å). Preliminary crystal data for the complex $[Cu(oxo)(bipy)]NO_3 \cdot H_2O$ suggest that the bonding mode is also similar [58].

The structure of $[Cu(cfH)(bipy)(Cl)_{0.7}(NO_3)_{0.3}](NO_3) \cdot 2H_2O$ [59] consists of the $[Cu(cfH)(bipy)(Cl)_{0.7}(NO_3)_{0.3}]^+$ cation, a nitrate anion and two water molecules. The structure is disordered, with the occupancy of the coordinated Cl^- and NO_3^- being 0.7:0.3. The copper ion displays a five-coordinate square-pyramidal coordination with two nitrogen donors

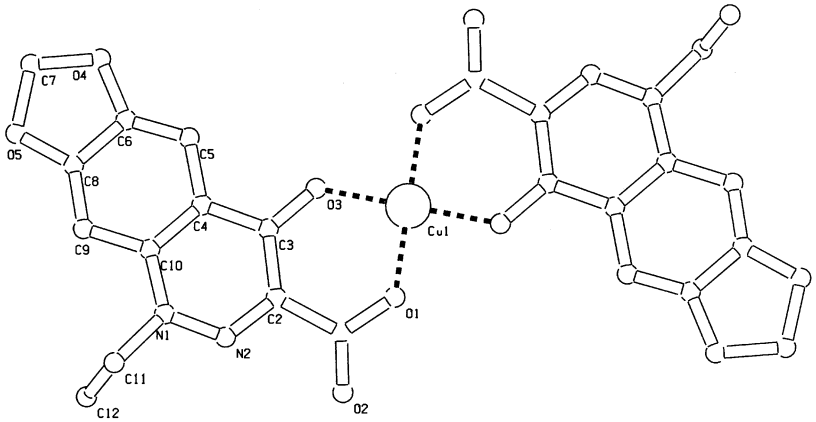


Fig. 6. Perspective view of $[Cu(cx)_2]\cdot 2H_2O$. Adapted from Ref. [55].

from 2,2′-bipyridine (bipy) (Cu(1)–N(4) = 1.977(3) Å, Cu(1)–N(5) = 1.994(4) Å), the 4-keto (Cu(1)–O(1) = 1.924(3) Å) and 3–carboxylate oxygen donors (Cu(1)–O(2) = 1.920(3) Å, and the disordered Cl^-/NO_3^- anion (Cu(1)–Cl(1) = 2.549(2) Å, Cu(1)–O(7) = 2.549(2) Å) occupying the fifth site.

All these mixed ligand complexes were prepared by mixing a water solution of $Cu(NO_3)_2$ and an ethanolic solution of N–N ligand (phen, bipy) with subsequent addition of an aqueous quinolone (nalH, cfH, oxolinic acid (oxoH)) solution.

The crystal structure and characterization of the mixed ligand complex of copper(I), triphenyl phosphine and nfH, $[Cu(PPh_3)_2(nfH)]ClO_4$, were published [44]. The copper ion displays a rather distorted tetrahedral geometry, being linked to two nfH oxygens (Cu(1)–O(2) = 2.0763(18), Cu(1)–O(1) = 2.074(2) Å) and two phosphorus atoms of triphenylphosphine ligand (Cu(1)–P(2) = 2.2389(8), Cu(1)–P(4) = 2.2472(8) Å).

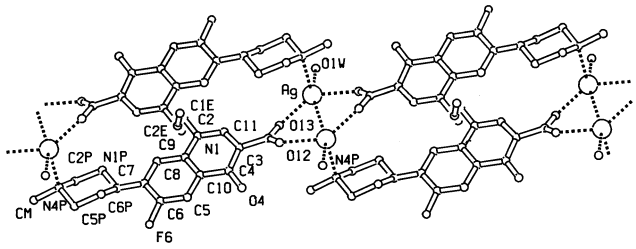


Fig. 8. Structure of the silver complex of pefloxacin. Adapted from Ref. [60].

2.2.9. Silver complex

A silver complex of pfH, $[Ag_2(pf)_2(H_2O)_2]\cdot 6H_2O$ [60] (Fig. 8) was isolated by dissolving a powdered silver complex in an aqueous ammonia solution. The bonding in this compound is substantially different from those described above. Two silver atoms are bridged by a pair of carboxyl groups from two pefloxacin molecules (Ag–O(12) = 2.329(3) Å, Ag–O(13) = 2.285(3) Å). The terminal piperazinyl nitrogen atom from another pefloxacin

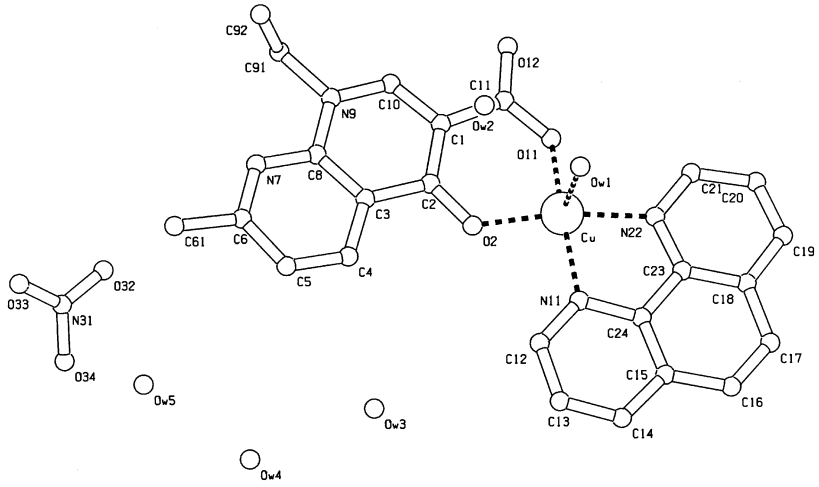


Fig. 7. Crystal structure of $[Cu(phen)(nal)(H_2O)]NO_3\cdot 3H_2O$. Adapted from Ref. [57].

molecule also coordinates to each silver atom (Ag–N(4P) = 2.431(3) Å). The silver coordination is completed by an oxygen atom from a water molecule (Ag–O(1W) = 2.454(4) Å). The two silver atoms are at a distance of 2.901(1) Å.

2.2.10. Zinc complexes

The complex $[Zn(nalH)_2(H_2O)_2](ClO_4)_2 \cdot 2H_2O$ [61] was prepared by mixing a solution of nalH in chloroform and an acetone solution of $Zn(ClO_4)_2$. The structure contains zinc in an octahedral ZnO_6 environment. The nalidixic acid bonds to metal in a β-diketonate-like chelation through carbonyl oxygen (Zn–O(3) = 2.048(5) Å) and carboxylic oxygen (Zn–O(1) = 2.083(5) Å). The octahedral coordination sphere of the dicationic complex is completed by two axial water ligands (Zn–O(4) = 2.116(5) Å).

Two zinc–norfloxacin complexes, $[Zn(nf)_2] \cdot 4H_2O$ and $[Zn(H_2O)_2(nfH)_2](NO_3)_2$ were isolated by hydrothermal synthesis [45]. The X-ray crystal structure of the first complex revealed that two nf anions are coordinated to the metal through ring carbonyl (Zn(1)–O(3) = 2.095(3) Å) and one of the carboxylate oxygens (Zn(1)–O(2) = 2.070(3) Å). Interestingly, the apical positions are occupied by two nitrogen atoms of piperazine rings (Zn(1)–N(1A) = 2.253(3) Å), resulting in the formation of a 2-D square grid with a nanosized hydrophobic tube cavity, that could be very useful for host–guest chemistry. In the second complex, the chelate bonding of the quinolone oxygen atoms to the metal is similar as in the first complex (Zn(1)–O(6) = 2.057(3), Zn(1)–O(5) = 2.028(3) Å), but the apical positions are occupied by two water molecules (Zn(1)–O(3) = 2.160(4)). The authors suggest that in a neutral or weakly basic solution, the nitrogen atom of the piperazine ring can take part in the coordination, while in the weakly acidic solution, this nitrogen is protonated and loses its coordination capacity. Authors also reported that both complexes show strong blue fluorescent emission and could be used as advanced materials for blue-light emitting diode devices.

The zinc complex $[Zn(cx)_2(DMSO)_2] \cdot 4H_2O$ [51] (Fig. 9) is structurally related to the nickel complex described above. The bonding distances are: Zn(1)–O(1) = 2.019(2) Å, Zn(1)–O(3) = 2.058(2) Å and Zn(1)–O(6) = 2.164(3) Å.

2.2.11. Cadmium complexes

The complex $[Cd_2(cx)_4(DMSO)_2] \cdot 2H_2O$ [62] was prepared from a DMSO solution of cxH and $CdCl_2$. Each dimer contains cadmium atoms bridged by two carboxylate oxygen atoms from two cx ligands generating a Cd_2O_2 ring. The metal environment is formed by two carboxylate and keto oxygen atoms from the cinoxacinate monoanions, one oxygen atom of the DMSO molecule and a carboxylate oxygen atom which acts as a bridging atom. One of the coordinated cinoxacinate molecules acts as a bidentate chelate and bridging ligand and the other as a bidentate chelate ligand. The distortion of the octahedron is not severe, with all Cd–O bond lengths in the range of 2.237(6)–2.350(7) Å.

The complex $[Cd_2(cx)_4(H_2O)_2] \cdot 10H_2O$ [63] (Fig. 10) was prepared by dissolving $[Cd_2(cx)_4(DMSO)_2] \cdot 2H_2O$ in hot water. Each cadmium atom is heptacoordinated. The metal environment consists of two keto oxygens (Cd(1)–O(3) = 2.319(6) Å, Cd(1)–O(3′A) = 2.341(5) Å) and two carboxylic oxygens (Cd(1)–O(1) = 2.216(6) Å, (Cd(1)–O(1′A) = 2.333(6) Å) from two different cinoxacinate monoanions. In addition, two carboxylate oxygen atoms from a third cinoxacinate ligand (Cd(1)–

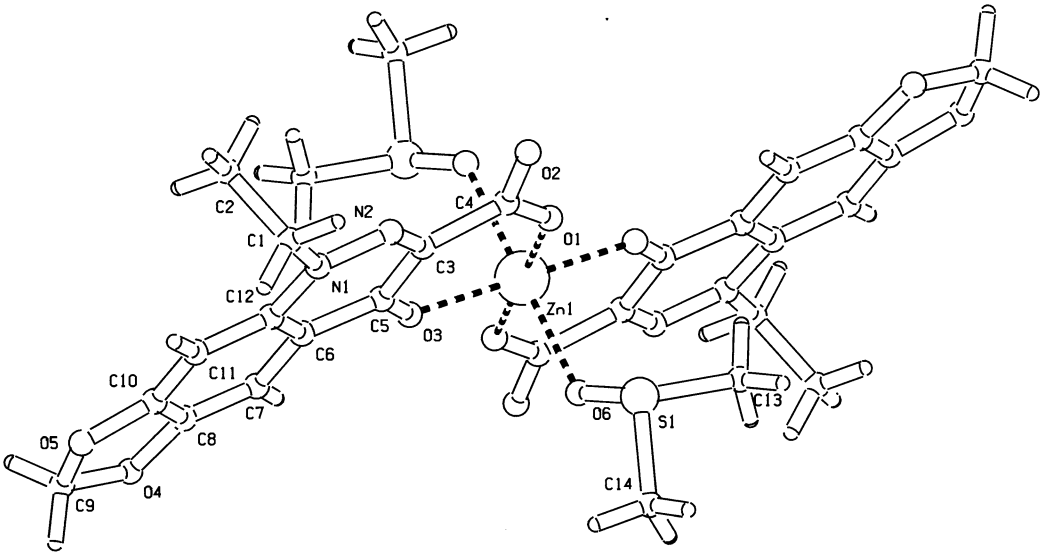


Fig. 9. View of $[Zn(cx)_2(DMSO)_2]$. Adapted from Ref. [51].

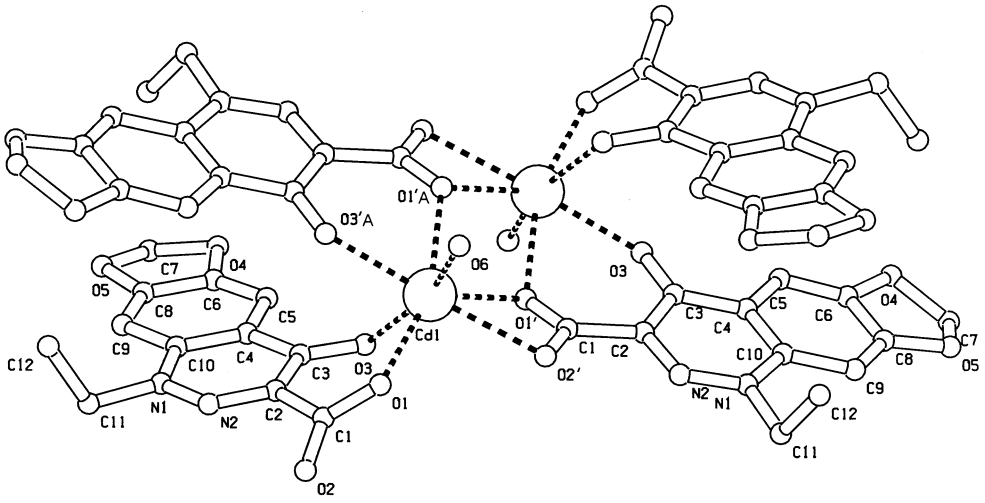


Fig. 10. View of $[Cd_2(cx)_4(H_2O)_2]$. Adapted from Ref. [63].

O(1′) = 2.357(6) Å, (Cd(1)–O(2′) = 2.526(7) Å) and a water molecule in the seventh position are coordinated to cadmium (Cd(1)–O(6) = 2.283(6) Å). Two of the cinoxacinate ions act as tridentate chelate bridging ligands and the other as a bidentate chelate ligand.

2.2.12. Cerium complex

The crystal structure of cerium(III) complex of cfH $[Ce(cf)_2(H_2O)_4]Cl(H_2O)_{3.25}(C_2H_5OH)_{0.25}$ was published [64]. The quinolone is again coordinated through 3-carboxyl and 4-keto oxygens and the coordination number of cerium is eight.

2.2.13. Short analysis of the metal–quinolone structures

The analysis of the structural data has revealed that in free quinolones the ring carbonyl carbon–oxygen distances are in the range from 1.246 to 1.276 Å and the distances between carbon and oxygen in carboxylic groups are in the range from 1.205 to 1.327 Å. Typically, if the carboxylic group is not dissociated one of the later bonds is much shorter (double bond) whereas the other bearing the hydrogen is longer (single bond). As indicated in paragraph 2.1. some quinolone molecules exist as the zwitter ions and the lengths of both carboxylic carbon oxygen bonds are thus nearly equal (ca. 1.25 Å).

The bonding of the metal to the quinolone oxygen atoms (described in Section 2.2) results in a slight lengthening of both ring carbonyl and carboxylic carbon–oxygen bonds. But of course, we should always be aware of other effects that could also influence the bond lengths (hydrogen bonding, etc.). It was also found that in the chelate bonding of the metal to ring carbonyl and one of the carboxylic oxygens both metal–oxygen bond distances are of similar lengths. Within the carboxylic group, the metal-coordinated oxygen expresses somehow longer carbon–oxygen distance than the noncoordinated oxygen. The most noticeable exception is $[Zn(nalH)_2(H_2O)_2](ClO_4)_2 \cdot 2H_2O$ where the later bond distance (1.299(9) Å) is much longer as the former (1.230(8) Å) [61].

There are no other distinctive differences in bond lengths between free quinolones and their metal complexes.

Some specific properties of metal–quinolone complexes are presented in Table 1. The most frequent are the copper(II) complexes of the quinolones which is probably not unusual in view of many copper(II) complexes with other ligands. Up to now the complexes of quinolones with 12 different elements were reported. The coordination numbers of the central atoms range from four to eight and the most frequently observed coordination polyhedron is octahedron. The mole ratios metal:quinolone are between 1:1 and 1:3.

We can conclude that the most common bonding observed for metal–quinolone complexes is the chelate bonding of the metal to ring carbonyl and one of the carboxylic oxygens. There are few complexes that are somehow different. In the complexes, $[Ag_2(pf)_2(H_2O)_2] \cdot 6H_2O$ [60] and $[Zn(nf)_2] \cdot 4H_2O$ [45], piperazine terminal nitrogen atom is also involved in the bonding to the metal. It was also found that few complexes (Ca, Mg, Cd) are dimeric [43,62,63]. We could hardly use any systematic approach (as 'Hard and Soft Acid and Base' concept) to classify the complexes. It seems that the differences in bonding of the reported complexes are mostly affected by different conditions used in synthesis.

2.3. Ionic compounds

All of these compounds were isolated from acidic solutions of quinolone and appropriate metal salts. These compounds generally consist of protonated qui-

Table 1
Selected data of the quinolone–metal complexes

Formula of the complex (reference)	Atoms involved in the bonding to the metal	Coordination number of the central atom
$[Mg_2(H_2O)_6(nfH)_2]Cl_4\cdot 4H_2O$ [43]	O(P), O(C), $O(H_2O)$	6
$[Ca_2(Cl)(nfH)_6]Cl_3\cdot 10H_2O$ [43]	O(P), O(C), Cl	7
$[B(C_{12}H_8F_2NO_3)(F)_2]$ [46]	O(P), O(C), F	4
$[B(C_{13}H_8Cl\ FNO_3)(C_4O_4H_6)_2]$ [47]	O(P), O(C), O(acetate)	4
$[VO(cfH)_2]SO_4\cdot 10H_2O$ [48]	O(P), O(C), O(vanadyl)	5
$[Fe(cfH)(nta)]\cdot 3.5H_2O$ [49]	O(P), O(C), O and N (nta)	6
$Na[Co(cx)_3]\cdot 6H_2O$ [50]	O(P), O(C)	6
$[Ni(cx)_2(DMSO)_2]\cdot 4H_2O$ [51]	O(P), O(C), O(DMSO)	6
$[Cu(cfH)_2Cl_2]\cdot 6H_2O$ [53]	O(P), O(C), Cl	6
$[Cu(cfH)(H_2O)_3]SO_4\cdot 2H_2O$ [54]	O(P), O(C), $O(H_2O)$	5
$[Cu(cx)_2]\cdot 2H_2O$ [55]	O(P), O(C)	4
$[Cu(cx)_2(H_2O)]\cdot 3H_2O$ [52]	O(P), O(C)	5
$[Cu(oflo)_2(H_2O)]\cdot 2H_2O$ [56]	O(P), O(C)	5
$[Cu(PPh_3)_2(nfH)]ClO_4$ [44]	O(P), O(C), $P(PPh_3)$	4
$[Cu(phen)(nal)(H_2O)]NO_3\cdot 3H_2O$ [57]	O(P), O(C), $O(H_2O)$, N(phen)	5
$[Cu(phen)(cx)(H_2O)]NO_3$ [58]	O(P), O(C), $O(H_2O)$, N(phen)	5
$[Cu(cfH)(bipy)(Cl)_{0.7}(NO_3)_{0.3}]$-$(NO_3)\cdot 2H_2O$ [59]	O(P), O(C), N(bipy, NO_3), Cl	5
$[Ag_2(pf)_2(H_2O)_2]\cdot 6H_2O$ [60]	O(C), N(piperazine), $O(H_2O)$	5
$[Zn(nalH)_2(H_2O)_2](ClO_4)_2\cdot 2H_2O$ [61]	O(P), O(C), $O(H_2O)$	6
$[Zn(nf)_2]\cdot 4H_2O$ [45]	O(P), O(C), N(piperazine)	6
$[Zn(H_2O)_2(nfH)_2](NO_3)_2$ [45]	O(P), O(C), $O(H_2O)$	6
$[Zn(cx)_2(DMSO)_2]\cdot 4H_2O$ [51]	O(P), O(C), O(DMSO)	6
$[Cd_2(cx)_4(DMSO)_2]\cdot 2H_2O$ [62]	O(P), O(C), O(DMSO)	6
$[Cd_2(cx)_4(H_2O)_2]\cdot 10H_2O$ [63]	O(P), O(C), $O(H_2O)$	7
$[Ce(cf)_2(H_2O)_4]Cl(H_2O)_{3.25}$-$(C_2H_5OH)_{0.25}$ [64]	O(P), O(C), $O(H_2O)$	8

O(P), ring carbonyl oxygen; O(C), carboxylic oxygen.

nolone cations and chlorometalate or simple inorganic anions.

The solubility of all ionic quinolone compounds is much greater than that of molecular complexes, which are only sparingly soluble.

2.3.1. Magnesium compound

In the magnesium adduct of cfH $(cfH_2)_2$-$[Mg(H_2O)_6](SO_4)_2\cdot 6H_2O$ [65] (Fig. 11), magnesium is not bonded to the quinolone molecule. The quinolone is protonated at the terminal nitrogen atom of piperazine residue. The hydrogen atom of the carboxylic group is hydrogen bonded to the carbonyl oxygen atom, thus preventing the bonding of the metal to this part of the molecule. The magnesium ion is coordinated by six water molecules forming a $[Mg(H_2O)_6]^{2+}$ cation with a nearly regular octahedral geometry. This is the only example of a metal–quinolone ionic compound reported with the water molecules coordinated to the metal.

2.3.2. Bismuth compounds

Two bismuth(III) compounds of cfH have been prepared $(cfH_3)(cfH_2)[BiCl_6]\cdot 2H_2O$ [66] (Fig. 12) and $(cfH_3)_2[Bi_2Cl_{10}]\cdot 4H_2O$ [67]. In the former, one of the cfH molecules is protonated at carbonyl oxygen and the terminal nitrogen of the piperazine residue, whereas the other is protonated only at the latter nitrogen atom. The charge of the isolated hexachlorobismuthate(III) anions is compensated by protonated cfH molecules. Due to their high charge, $[BiCl_6]^{3-}$ anions are not very common as the formation of polynuclear anions seems to be preferred [68–70].

In the latter compound Bi(III) ions are coordinated by chloride ions forming dinuclear $[Bi_2Cl_{10}]^{4-}$ anions. Both quinolone molecules are doubly protonated in this compound.

2.3.3. Iron compound

Four chloride ions are coordinated to the iron(III) ion, forming a slightly distorted tetrahedron in the enrofloxacin compound $(erxH_3)[FeCl_4]Cl$ [71] (Fig. 13). The carboxylic group is not deprotonated and a carbonyl oxygen O(1) is protonated; the consequence is that this part of the molecule is unable to bind to the metal. The quinolone molecule is additionally protonated at the nitrogen atom N(24), thus its charge is +2.

2.3.4. Platinum compound

The formula of the platinum(II) compound of pefloxacin is $(pfH_2)_2$ $[PtCl_4]\cdot 2H_2O$ [72]. The coordination geometry of platinum is square planar. The terminal nitrogen atom of the pefloxacinum cation is protonated. An intramolecular hydrogen bond between the carboxylic hydrogen and the keto ring oxygen forms a pseudo six-membered ring.

2.3.5. Copper compounds

Four chloride ions are coordinated to the copper(II) ion, forming a rather distorted tetrahedron in $(nfH_3)(nfH_2)[CuCl_4]Cl\cdot H_2O$ [35] (Fig. 14). There are two nonequivalent norfloxacin molecules in the asymmetric unit. In the first (indicated by A), the carbonyl O(1) and piperazine nitrogen N(24) are both protonated. In the second (indicated by B), only N(24) is protonated. There are distinctive layers of quinolone molecules in the structure, and the distances between

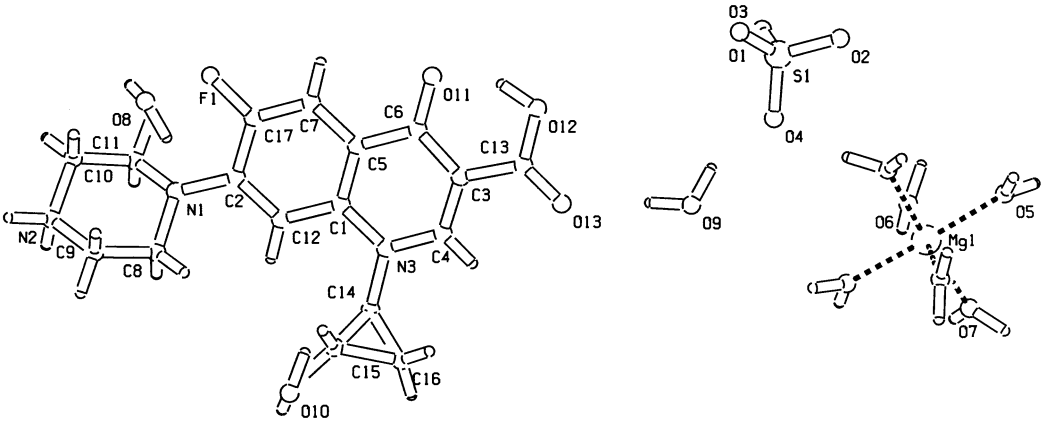


Fig. 11. View of $(cfH_2)_2[Mg(H_2O)_6](SO_4)_2 \cdot 6H_2O$. Adapted from Ref. [65].

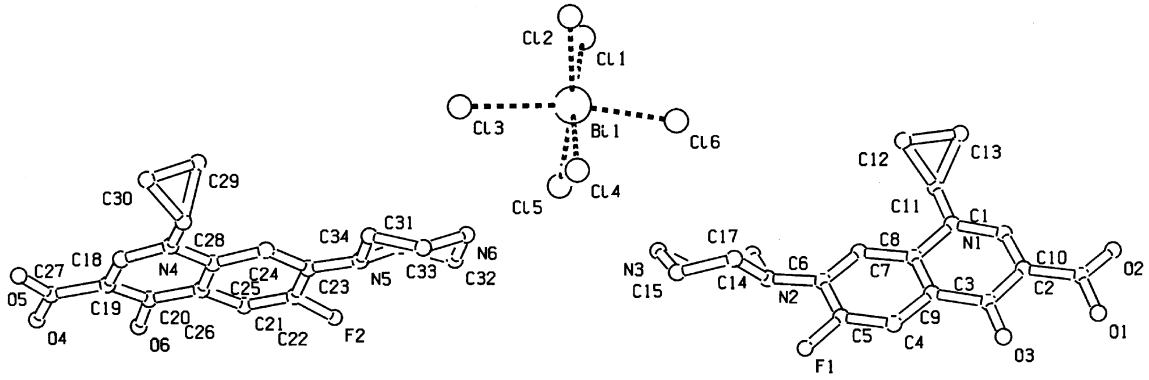


Fig. 12. View of $(cfH_3)(cfH_2)[BiCl_6] \cdot 2H_2O$. Adapted from Ref. [66].

aromatic rings in the neighboring layers are around 3.5 Å; thus, interactions between π-electronic systems are possible (Fig. 15).

The second compound of quinolone and copper(II) $(kinoH_3)[CuCl_4] \cdot H_2O$ [71] is similar to the first. It contains tetrachlorocuprate(II) ions and protonated quinolone molecules. A distinctive pattern was observed in this compound (Fig. 16)—two neighboring quinolone molecules form a dimeric pair via carboxylic groups, which is otherwise typical for free carboxylic acids.

2.3.6. Zinc compounds

The zinc compound $(nfH_3)(nfH_2)[ZnCl_4]Cl \cdot H_2O$ [35] is isotypic to the copper–norfloxacin compound (see above).

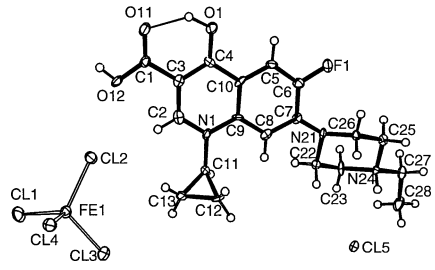


Fig. 13. View of $(erxH_3)[FeCl_4]Cl$. Reproduced with permission from Elsevier Science—Ref. [71].

The zinc compound of cfH with the formula $(cfH_3)[ZnCl_4] \cdot H_2O$ [73] is also very similar to the zinc compound of norfloxacin. There are distinctive layers of quinolone molecules in the structure which are cross-linked through extensive hydrogen bonding.

The inspection of the bond lengths in the isolated ionic type complexes have revealed the following facts:

- the quinolone molecules could be mono- or doubly protonated,
- in monoprotonated species only the terminal nitrogen atom of piperazine ring is protonated,
- in doubly protonated species additionally the ring carbonyl oxygen is protonated. If this is the case, this carbon–oxygen bond is substantially lengthened (1.30–1.33 Å).

3. Thermal analyses

Only a few references are available on the thermal properties of free quinolones [74–76]. It has been found that polymorphism is very common for quinolones. The polymorphs show different melting points, solubilities, chemical reactivity and stability.

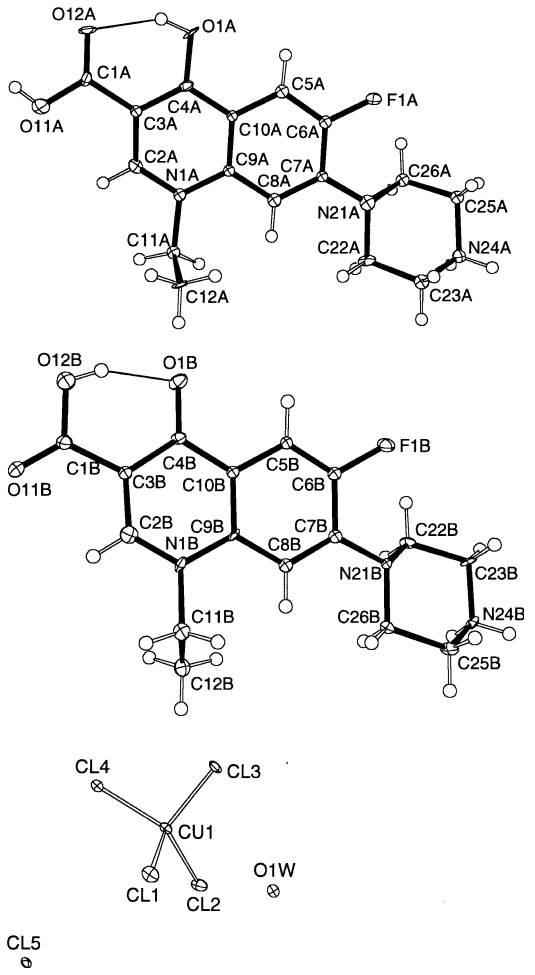


Fig. 14. View of $(nfH_3)(nfH_2)[CuCl_4]Cl\cdot H_2O$. Reproduced with permission from Elsevier Science—Ref. [35].

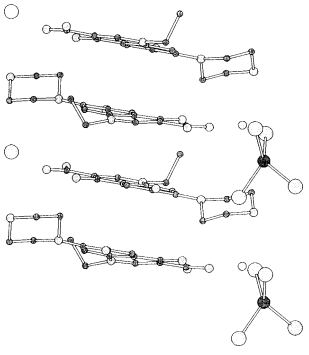


Fig. 15. Distinctive layers of quinolone molecules in $(nfH_3)(nfH_2)$-$[CuCl_4]Cl\cdot H_2O$. Reproduced with permission from Elsevier Science—Ref. [35].

The formation of cyclodextrin (CD)–norfloxacin complexes has also been studied by thermal and other methods [77]. It has been proposed that quinolone is incorporated in the CD framework.

The thermal behaviour of three metal complexes, $Na[Co(cx)_3]\cdot 10H_2O$, $Ni_2(cx)_3(ClO_4)\cdot 8H_2O$ and $Cu(cx)_2\cdot H_2O$, has been studied by thermogravimetry (TG) and differential thermoanalysis (DTA) [78]. It was found that the complexes decompose in two steps: dehydration is followed by decomposition of the anhydrous complexes to metal or metal oxide. Similar behaviour was also found for Zn(II), Cd(II) and new Co(II) complexes [79].

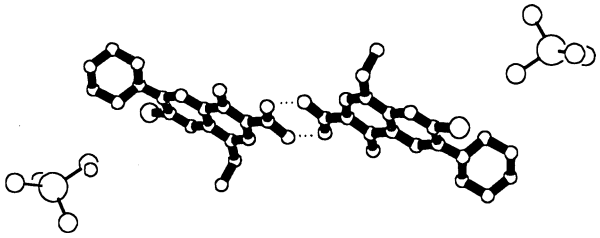


Fig. 16. Schematic view of two neighboring quinolone molecules in $(kinoH_3)[CuCl_4]\cdot H_2O$ forming a dimeric pair via carboxylic groups (intermolecular hydrogen bonds). Reproduced with permission from Elsevier Science—Ref. [71].

The thermal properties of $[Cd_2(cx)_4(DMSO)_2]\cdot 2H_2O$ and $[Cd(cx)_2(H_2O)]$ were compared [62]. The TG curves of the complexes show three well-separated thermal events. The first (100–150 °C) corresponds to the elimination of two water and two DMSO molecules from the first compound, and to the elimination of only the water molecule from the second compound.

It was found that it is possible to obtain anhydrous complexes from the hydrated mixed ligand complexes Cu–nal–bipy (phen) by heating the samples to 110 °C [57]. It was proposed that the mixed ligand complexes are more stable than the $Cu(nal)_2$ complex.

A series of mixed ligand complexes of the types Cu–oxo–bipy (phen) and Cu–cx–bipy (phen) were also characterized by the TG method [58]. It was observed that in these systems it is also possible to obtain anhydrous complexes by heating the samples up to 120 °C. These anhydrous complexes are stable up to temperatures near 260 °C.

The thermal study of the mixed ligand complexes of the type Zn–nal–phen (bipy) has revealed that the decomposition is dependent on the diammine present in the structures [80]. The difference suggests that the diammine strongly changes the acid–base behaviour of the metal ion, becoming harder with bipy than with phen. This means that the complexes with bipy have a stronger affinity for oxygen than the complexes with phen.

The thermal behaviour of copper–cfH compounds $[Cu(cf)_2Cl_2]\cdot 6H_2O$ and $(cfH_3)(cfH_2)[CuCl_4]Cl\cdot H_2O$ was studied by TG, dynamic scanning calorimetry (DSC) and evolved gas analysis (EGA) methods [81,53]. In the first compound the dehydration is followed by decarboxylation, whereas in the second the dehydration is followed by pyrolysis with simultaneous evolution of water, hydrochloric acid and carbon dioxide.

The thermal properties of bismuth compounds of cfH [66], copper and zinc complexes of norfloxacin [35] were also studied. In each of these compounds, dehydration is followed by complex pyrolysis. It was possible to determine the water content of the compounds, though it was also found that not all water molecules are equivalently bonded. The weaker-bonded water molecules could be lost in metal–quinolone complexes even by drying in air at room temperature (r.t.) or by drying in vacuo [53].

A study of the thermal properties of magnesium compounds of quinolones $(cfH_2)_2[Mg(H_2O)_6](SO_4)_2 \cdot 6H_2O$, $[Mg(cf)_2]\cdot 4H_2O$ and $[Mg(nf)_2]\cdot 4H_2O$ was also performed [82]. The derivative thermogravimetry (DTG) curves for dehydration reactions are all split into two peaks, suggesting the presence of two differently bonded water molecules in the structure. The final decomposition product was MgO for the first compound and MgF_2 for the other two compounds. Due to these observations, the authors proposed that Mg(II) is coordinated to the quinolone in $[Mg(cf)_2]\cdot 4H_2O$ and $[Mg(nf)_2]\cdot 4H_2O$. Recently the same authors have also studied the thermal behaviour of Mg(II), Zn(II) and Co(II) compounds with cfH. It was found that the complexes decompose in two steps, dehydration and pyrolytic decomposition of the anhydrous complexes to form metal oxide or metal fluoride [83].

In conclusion, the thermal analyses of metal–quinolone complexes have shown that in general they decompose in two steps. The first step is dehydration at lower temperatures, which is followed by pyrolytic decomposition at higher temperatures. The final product could be the metal, metal oxide or metal fluoride.

4. Infrared spectroscopy

Infrared (IR) spectra of quinolones are quite complex due to the presence of numerous functional groups in the molecules. The IR spectrum of anhydrous norfloxacin was assigned [75]. The valence vibration of the carboxylic stretch $\nu(C{=}O)_c$ was found at 1725 cm^{-1} and the pyridone stretch $\nu(C{=}O)_p$ at 1628 cm^{-1}. It was found that the IR spectrum of norfloxacin dihydrate does not exhibit a well-defined carboxylic stretch.

The IR spectra of cfH and cfH hexahydrate do not have a $\nu(C{=}O)_c$ absorption [32]. According to the crystal structure of $cfH\cdot 6H_2O$, the authors have concluded that the carboxylic group is deprotonated and the molecule exists in zwitterionic form. It is known that ionic carboxylates [84] show no carbonyl stretching at about 1700 cm^{-1}, but have two characteristic bands in the range of 1650–1510 cm^{-1} and 1400–1280 cm^{-1} that could be assigned as $\nu(O–C–O)$ asymmetric and symmetric stretching vibrations. It is very difficult to unequivocally assign these vibrations in the spectra of cfH due to the numerous other bands present in these regions; however, the combination of IR and Raman spectroscopies enabled the authors to propose some possible assignments. The asymmetric stretching vibrations—$\nu(O–C–O)_a$ were found in $cfH\cdot 6H_2O$ at 1578 and at 1589 cm^{-1} in an anhydrous cfH, whereas the corresponding symmetric stretching vibrations—$\nu(O–C–O)_s$ were found at 1380 cm^{-1} ($cfH\cdot 6H_2O$) and 1376 cm^{-1} (cfH). It was also found that after heating of both samples to higher temperatures (270 °C), a peak at 1727 cm^{-1} appeared in the spectra. It seems that the role of a carboxylic group in the structure is changed during heating, though the mechanism of this phenomenon was not explained.

Several authors have used IR data to characterize metal compounds of quinolones, mainly focusing only on the most typical vibrations. It was found that when the metal is bonded to quinolone 4-oxo and carboxylic oxygens there is no $\nu(C{=}O)_c$ absorption in the spectra, whereas $\nu(C{=}O)_p$ is shifted upon bonding. Also, some new absorptions often appeared in the spectra of complexes [53,54,82,85–88].

The range of nalH metal complexes was studied by IR spectroscopy [89]. The authors have calculated the differences between $\nu(O–C–O)_a$ and $\nu(O–C–O)_s$ and have concluded that in some complexes nalH acts as a bridging ligand (Ca, Mg, Mn, Ni, Zn, Cd), whereas in other complexes (Co, Fe, Cu, Pd) it acts as a chelate ligand.

Mendoza-Díaz et al. have studied mixed ligand complexes of the type Cu (Zn)–nal–phen (bipy) [57,58,80]. The solid state IR spectra showed bands corresponding to both ligands. In the region 1800–1300 cm^{-1}, bands of 4-oxo and 3-carboxylate appeared. The results suggest that the bonding mode of the nalidixate ion should be the same in all complexes.

Ruiz et al. have studied mixed ligand complexes of the type M–cx–DMSO ($M^{2+} = Cu^{2+}$, Cd^{2+}, Ni^{2+}, Zn^{2+}) [51,55,62,63]. They have assigned the most significant bands: 3600–3200 cm^{-1} (ν(O–H)), 1650–1600 cm^{-1} ($\nu(O–C–O)_a + \nu(C=O)_p$), which appeared in all complexes.

The infrared spectrum of $[Fe(cfH)(nta)]\cdot 3.5H_2O$ [49] is very complex due to the additional absorptions of nta. The authors were not able to assign the difference $\nu(O–C–O)_a - \nu(O–C–O)_s$ from which the bonding mode of the carboxylic group could be predicted. They have concluded that the bonding mode of more complex ligands containing carboxylate groups bonded to the metal could not be unambiguously established on the basis of IR spectra alone.

Lecomte et al. [90,91] have studied the interactions of magnesium and different quinolones (sparfloxacin, pefloxacin) by IR spectroscopy. They have recorded the IR spectra in D_2O solutions at different pH values. At pH 4, $\nu(C=O)_p$ was found at 1632 cm^{-1} and $\nu(C{=}O)_c$

at 1698 cm^{-1} for sparfloxacin. At pH 12, $\nu(C=O)_p$ was at 1622 cm^{-1}, $\nu(O–C–O)_a$ was at 1580 cm^{-1} and $\nu(O–C–O)_s$ was at 1400 cm^{-1}. In the presence of Mg(II) (pH 7.4) the bands were shifted, so they have suggested that magnesium is located between the keto and carboxylate oxygens of the quinolone. With regard to the different shifts of pfH and sparfloxacin, they have suggested that the structures of the magnesium complexes of these drugs are different.

The combination of thermal methods and IR spectroscopy was used to study the properties of $[Cu(cfH)_2Cl_2]\cdot 6H_2O$ and $(cfH_3)(cfH_2)[CuCl_4]Cl\cdot H_2O$ [81]. The heating of the samples changed the positions and the appearance of some bands, which is probably a consequence of changes in the coordination sphere of the metal. Additionally, water molecules or hydrochloric acid molecules are removed from the structures by heating and some hydrogen bonds are broken or rearranged; the result is a shift of band frequencies towards higher wavenumbers. The most notable fact is that in $[Cu(cfH)_2Cl_2]\cdot 6H_2O$, apart from shifts of bands that could be explained by a different hydrogen bonding scheme, a peak at 1723 cm^{-1} appeared in the spectrum after heating. Obviously, then, the role of the carboxylic group is altered in the structure.

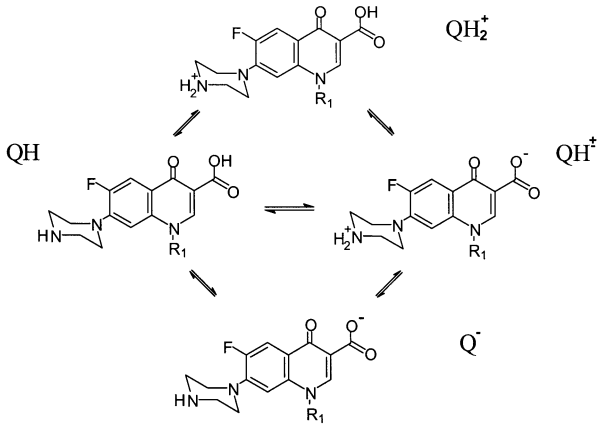
In all ionic type compounds with known X-ray structures, similar types of IR spectra appeared [35,66,71]. The carboxylic group is involved in hydrogen bonding, and in all examples it was possible to assign the intense band in the 1714–1663 cm^{-1} region as $\nu(C=O)_c$. The position of $\nu(C=O)_p$ was not changed according to the metal complexes described above, and appeared at around 1630 cm^{-1}.

5. Protolytic equilibria of quinolones and their metal compounds. Determination of stability constants and the stoichiometry of complexes

The protolytic equilibria of quinolones were extensively studied. Different methods (potentiometric measurements, UV–vis spectroscopy, polarography, fluorescence spectroscopy and NMR spectroscopy (the results of the latter technique are collected in Section 7)) were used to evaluate the macro- and microconstants of the species present in solution [59,92–98]. The differences in the determined values are probably due to the methodology and conditions used.

Nalidixic acid has a single protonation constant ($pK_a \approx 6$) in the pH range of 2–9 [85,92,93,99,100]. It is protonated in a strong acid solution to form a naphthyridinium cation with a pK_a of -0.86 [101]. The drug exists in its undissociated form over a pH range of 1.6–3.6.

Fluoroquinolone analogues with the piperazinyl group in the 7-position contain two relevant ionizable



Scheme 3. Protolytic equilibria in fluoroquinolone drugs water solutions.

functional groups. The protolytic equilibria of fluoroquinolone analogues are expressed in Scheme 3. These molecules can exist in four possible forms: an acidic cation H_2Q^+, a neutral nonionized species HQ, an intermediate zwitterion $HQ^{\pm}$ and a basic anion Q^-, depending on the pH. At low pH values, both the 7-piperazinyl group and 3-carboxyl group are protonated, whereas at high pH values, neither is protonated. The carboxyl group is normally a stronger acid than the ammonium group, the reason being that the neutral nonionic form is spontaneously rearranged to the zwitter ion. Two macroscopic dissociation constants can be determined for fluoroquinolones. The first (K_{a1}) applies to the 3-carboxyl proton and the second (K_{a2}) to the 7-piperazinyl proton. The pK_{a1} of cfH and nfH was found at around 6. The decrease in acidity of the carboxylic group compared with benzoic acid (pK_a 4.2) is explained by the intramolecular hydrogen bond to the keto oxygen [102]. The pK_{a2}, which is due to the presence of an ionizable proton on the external piperazinyl nitrogen, was found at around 8.5. A further protonation step at very acidic conditions was also found ($pK_a \approx 0$), which could be explained by the deprotonation of the keto group [103]. It is interesting to note that in non-aqueous solutions, the pK values of nfH were found at 0.74 and 8.26, respectively [85].

Numerous authors have also determined the stability constants of metal–quinolone complexes.

The stability constants for the binding of nalH by several divalent metal ions were determined by UV–vis and fluorescence spectroscopies [92]. The magnitudes of the formation constants support the physiological significance of the 1:1 complexes and the lack of importance of the 2:1 (drug:metal) complexes.

The interaction of nalH with Al(III), Mg(II) and Ca(II) was studied by UV–vis spectroscopy [99]. The stoichiometry of the nalH–Al complex was estimated to be 3:1 by the method of continuous variations. Efforts

directed at determining formation constants of nalH–M complexes have been unsuccessful.

It was also found that ternary complexes are formed between guanosine-5′-monophosphate (GMP)–copper(II)–nal [100]. This supports the suggestion that the metal ion-mediated binding of nalH to single-stranded deoxyribonucleic acid (DNA) in vitro also shows a preference for guanine residues. These results imply the initial formation of a mixed ligand complex between the drug and DNA (with the metal ion acting as a bridge).

The study of complexation equilibria of the nalidixate and cinoxacinate with $[Cu(phen)]^{2+}$ and $[Cu(bipy)]^{2+}$ was also performed [104]. The study indicates that the stability of these types of complexes is strongly related to the metal environment. These results suggest that inside the living cells a possible interaction of quinolone with some metal ion will be strongly controlled by the type of ligand bound to the cation.

The complexation of quinolones (nalH, cfH) and iron(III) was studied by UV–vis spectroscopy [105,106]. The author proposed the formation of $Fe(quinolone)_2$ complexes. The described procedure could also be used for the determination of trace amounts of iron.

The formation constants of iron(III)–fluoroquinolone analogues (cfH, nfH, enoxacin, ofloxacin) were determined by UV–vis spectroscopy [85]. The authors established the stoichiometric compositions of the complexes by Job's method of continuous variations, which indicate the formation of a 1:1 complex. The formation constant of the nfH–Fe(III) complex was lower than that found by Issopoulos [105,106].

The stability constants of metal complexes and quinolones were determined by potentiometry and spectrophotometry [107]. The values of the stability constants for Al(III), Mg(II) and Ca(II) complexes were $Ca < Mg << Al$. The stability constants of lomefloxacin complexes with divalent transition metal ions followed the Irving–Williams series [108] ($Mn < Fe < Co < Ni < Cu > Zn$).

It was found that ofloxacin reacts with Cu(II) to form complexes 1:1 (drug:metal) (pH 4.00), 2:1 (pH 7.02) and 3:1 (pH 8.30) [109]. The 1:1 and 3:1 complexes were confirmed by a polarographic method.

The ability of Al(III) to form complexes with nfH was investigated by potentiometry and fluorescence spectroscopy [110]. The stability constants of numerous complexes formed in the system were calculated.

The interaction of Co(II), Ni(II), Cu(II) and Zn(II) with cxH has been studied by means of pH-metric, spectrophotometric and ESR methods [52]. In all systems, complexes with different stoichiometric ratios, in which cxH acts both as a neutral and a deprotonated ligand, are formed. The anomalous sequence of the stepwise stability constants observed for Co(II) and Ni(II) suggests changes in stereochemistry when $Co(cx)_2$ and $Ni(cx)_2$ are formed. For the Cu(II) system, the sequence in the stepwise stability constants indicates the preferential formation of the $[Cu(cx)]^{+}$ monocomplex.

The formation constants of nfH and cfH–Cu(II) complexes were determined by potentiometric titrations [59]. The addition of cfH to metal ions resulted in the formation of ML and ML_2 complexes. The overall and stepwise formation constants were calculated and the speciation plots were constructed.

Some metal (Ca(II), Co(II), Ni(II), Cu(II), Zn(II), Al(III), Fe(III)) complexes of cfH were studied by potentiometric and spectroscopic methods in solution [94]. It was found that different protonated complexes are formed before precipitation in the systems studied. In the more acidic region a 1:1 complex is favored, whereas a 1:2 (metal:drug) complex prevailed at higher pH values. As the coordination of the second ligand is more favored than that of the first, it seems probable that the 1:1 complex is more distorted. Job's technique confirmed that the 1:1 = Cu:cfH complex is the major component of the system at pH 2.5. Iron and aluminium form the complexes with a 1:3 metal to ligand ratio.

The interaction of nfH with di- and trivalent cations was studied by potentiometric titrations [111]. 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 nfH in dogs were investigated. The data indicated the likely formation of a 1:2 metal ion–quinolone complex at the pH of the upper gastrointestinal tract from which the fluoroquinolones are absorbed.

The complex formations of different quinolones with various metal cations were investigated by pH-titrations and NMR spectroscopy [86]. The order of stability constants among trivalent metal cations was Fe(III) > Al(III), and that among divalent metal cations was $Cu(II) > Fe(II) > Zn(II) > Mg(II) > Ca(II)$. It was proposed that quinolones interact with Al(III) in the stomach, but with Mg(II) in the intestines when coadministered with antacid containing Al(III) and Mg(II).

Alkaysi et al. have used Job's technique and molar ratio methods to determine the stoichiometry of metal–nfH complexes [112]. The determined ratios were nfH:Al(III) = 2:1 and nfH:Mg(II) = 3:1. It has been reported that the formation of a complex with Al(III) enhanced the water solubility of the drug.

The UV–vis experiments employing the continuous variation method showed that the typical stoichiometry for the Al(III)–cfH chelate is 1:1 [13]. Steric hindrance by the first cfH molecule may significantly affect chelation of subsequent molecules.

Job's method was also used to determine the stoichiometry of the mixed ligand complexes Cu–bipy–nal and Cu–phen–nal [57]. The study was possible owing to the fact that in solution, $[Cu(bipy)]^{2+}$ or $[Cu(phen)]^{2+}$ exist practically without any dissociation. It was found that the $[Cu(bipy)]^{2+}$:nal and $[Cu(phen)]^{2+}$: nal ratios are 1:1. Similar results were also found in Cu-bipy (phen) complexes with oxoH and cxH [58].

The complexation of nalH and nine fluoroquinolone drugs with Mg(II) and Ca(II) was studied by spectrofluorometry [113]. Scatchard plots were used to determine the stoichiometry of ligation. Based on these results, a 2:2 ratio was proposed involving coordination at the carboxylic acid, the adjacent 4-keto group and the terminal nitrogen of the piperazine ring.

The complexation of lomefloxacin with five metal ions (Al(III), Ca(II), Mg(II), Bi(III) and Fe(III)) was studied [114]. The stability constants and stoichiometries were determined by measuring the change of aqueous solubility of lomefloxacin as a function of metal ion concentration. It was established that calcium forms a 1:1 complex, magnesium, bismuth and iron a 1:2 (metal:drug) complex and aluminium a 1:3 complex.

To sum up, the pK_a values of most free quinolones have been determined by various methods. Additionally the stability constants of several quinolone–metal complexes have been reported. It is hard to make some general conclusions but it seems that the values of stability constants with divalent transition metal ions follow the Irving–Williams series [108].

6. UV–vis spectroscopy studies

Apart from the data used for the calculation of stability constants and determination of stoichiometry, the changes in the UV part of the quinolone spectra were also studied. The UV spectra of quinolones are pH dependent. In the representative example cfH, three main maxima at 274–278, 324 and 340 nm are observed, which are all shifted at different pH values. The changes can be attributed to the extent of ionization of the carboxylic group. Numerous authors have also studied the changes in spectra upon the addition of metal ions [15,52,82,85,92,99,100,105–107,109,112]. Some authors have also interpreted the d–d transitions of some metal–quinolone complexes.

The d–d transitions were studied in the complexes Cu–phen–oxo (nal, cx) and Cu–bipy–oxo (nal, cx) [58]. In the first type of complexes the λ_{max} appears between 636.6 and 632.9 nm. In the second group the λ_{max} appears between 626.7 and 617.3 nm. From these results it is clear that the main effect on the average ligand field is caused by the diammine, and that all antibacterial agents used have almost the same contribution to the average ligand field.

The diffuse reflectance spectra of $[Ni(cx)_2(DMSO)_2]\cdot 4H_2O$ and $Ni(cx)_2\cdot 2H_2O$ show d–d bands and a metal ligand charge transfer band. The d–d bands were assigned as electronic transitions of an Ni(II) ion in an octahedral environment which was in agreement with the crystal structure of $[Ni(cx)_2(DMSO)_2]\cdot 4H_2O$ [51]. The diffuse reflectance spectrum of $Na[Co(cx)_3]\cdot 10H_2O$ together with the magnetic moment (4.97 BM) is consistent with the presence of a high-spin octahedral cobalt(II) ion [63]. Ligand field parameters were calculated giving $D_q = 854\ cm^{-1}$ and $\beta = 0.98$. The spectrum of $Cu(cx)_2\cdot 2H_2O$ is in agreement with a square planar coordination around copper(II) confirmed by X-ray crystallography [63].

7. NMR spectroscopy

The ^1H and ^{13}C-NMR spectra of nfH were recorded in deuteroacetic acid and the resonances were assigned [75]. Norfloxacin solutions were also titrated with DCl and NaOH and the pK_a values were determined [103]. Three pK_a values were determined, which correspond to deprotonation of the carboxylic group (pK_a 0.6), pyridone nitrogen (pK_a 6.9) and external piperazine nitrogen (pK_a 9.25). These assignments were corrected according to the ^{13}C-NMR study of nfH in 3 M DCl solution [34]. The authors have suggested that the pK_a at 0.6 is that of the keto oxygen atom and the pK_a at 6.9 is that of the carboxylic group.

A series of isolated 6,7- and 7,8-disubstituted quinolones was also characterized by ^1H spectroscopy [115].

The acid–base properties of seven fluoroquinolone derivatives were studied by NMR spectroscopy [93]. The basicities of the functional groups were quantified in terms of macroconstants, and also at the submolecular level in terms of microconstants. The microspeciation was also presented.

^1H-NMR was used to probe the interactions experienced by cfH following uptake into large unilamellar liposomes [116]. It was shown that cfH is located in the aqueous interior of the liposomes and is self-associated in the form of small stacks. cfH does not precipitate, even though its intraliposomal concentration can exceed its solubility in aqueous solutions by almost two orders of magnitude.

Interaction between nfH and β-CD in solution was characterized by ^1H-NMR [77]. It was suggested that the piperazine group of nfH is that part of the molecule which is bound inside the β-CD cavity.

The complexation of lomefloxacin, nfH and the metal ions (Al(III), Mg(II) and Fe(III)) was studied with ^1H and ^{13}C-NMR in D_2O solutions [38]. Studies using Al(III) resulted in the appearance of three or four additional peaks with the addition of a metal ion to the drug solution due to slow exchange between the

complexed and the free drug. Two complexes were proposed to have stoichiometries of 2:1 and 3:1 (drug:-metal) based on peak widths and variable temperature studies. The crystal structure of free lomefloxacin was used together with NMR data on the aluminium complexes in molecular modeling of the lomefloxacin–Al (3:1) complex. The authors suggested that three lomefloxacin molecules are coordinated to aluminium through carboxylic oxygen and 4-keto oxygen.

Benigno Macías Sánchez et al. reported that only minor shifts were found in the ^{1}H-NMR spectra of compounds isolated from systems containing quinolone (cfH, ofloxacin) and metal (Al(III), Mg(II), Ca(II), Fe(II)) [87]. Small shifts could be due to the change in the counteranion or to a different association of the quinolone molecules. No functional group seemed to be lost or modified in the prepared compounds.

^{19}F-NMR was used to measure the affinity of magnesium for six fluoroquinolones [90,91]. It was proven that magnesium is located between the keto and the carboxylate groups. The binding constants for the 1:1 Mg^{2+}–drug complexes were determined. Sparfloxacin and pfH, with affinity constants (K_a) of $(10.1 \pm 0.6) \times 10^2$ and $(21 \pm 1) \times 10^2$ M^{-1}, respectively, were the least and the most bound.

The extent of pfH stacking depends strongly on pH and was studied by ^{1}H, ^{13}C and ^{19}F-NMR [117]. The authors concluded that pfH is monomeric at the concentration 10^{-4} M. At such a concentration of pfH and high cation–quinolone ratios, 1:1 and 1:2 (drug:cation) complexes with magnesium and calcium form at pH 7.4 [118]. It was supposed that the binding sites are first the carbonyl and carboxylic groups, then the terminal nitrogen piperazinyl atom. When the pfH concentration is increased, the affinity of this drug increases. This is due to the formation of a 2:2 complex which enhances the stacking of this fluoroquinolone.

^{19}F-NMR spectra show that in the absence of magnesium, pfH binds poorly to DNA and preferentially to single-stranded rather than to double-stranded DNA [119]. The data show that the quinolone ring of pfH has little mobility in the ternary complex. In this complex, pfH could be bound to two magnesium ions, each cation acting as a bridge between this molecule and a phosphate group of the DNA backbone.

The NMR titration experiments have revealed that the binding ability of levofloxacin (this molecule is an *S*-enatiomer of ofloxacin, see Scheme 2) toward Al(III) is much stronger than that of cfH and lomefloxacin at pD 2.5 [120]. In contrast to the complexation with Al(III), the binding of these drugs with other metal ions such as Ca(II) and Mg(II) is much weaker. NMR signals have shown no appreciable downfield shift by the addition of Ca(II) and Mg(II). Based on these results, it was concluded that the fluoroquinolone antimicrobials examined in the present study at pD 2.5 exist as stable complexes in the presence of Al(III).

The coordination mode of quinolone DR-3862 in the presence of Al(III) was studied by ^{13}C-NMR [86]. The chemical shift changes of the carbon atoms near the carbonyl group of DR-3862 were very large, and the bonding of Al(III) to the carbonyl and carboxylic groups was proposed.

The participation of the carbonyl and carboxylic groups in the chelating reaction of different quinolones was also confirmed by ^{13}C-NMR measurements of the Al(III) and Mg(II) complexes [107].

The ^{13}C solid state NMR spectrum of $[Cd_2(cx)_4(DMSO)_2]\cdot 2H_2O$ revealed that each carbon atom (C_4, C_{16}, C_{15}, C_{DMSO}) shows two signals as a consequence of the two cxH ligands being coordinated in different ways to cadmium in the asymmetric unit [62].

The ^{13}C-NMR study was performed in Zn–nal–phen (bipy) systems [80]. The authors proposed that the nalidixate is coordinated through the carbonyl and carboxylate groups in a trigonal bipyramidal geometry (with the diammine in the equatorial position, the drug bonded to one equatorial and one axial position and the remaining position for the anion as chloride or nitrate).

The complexation of Cu(II) and nalH was studied by ^{13}C-NMR [121]. It was found that the site of binding depends upon the nature of the other ligands present in solution. In the absence of added ligands, chelation via the 3-carboxylate group was observed, while interaction with $[Cu(phen)]^{2+}$ is via chelation with both carboxylate and 4-oxo groups.

From the ^{13}C-NMR study in the Cu(II)–cfH system [59] it appears that in an acidic solution, the primary source of interaction with the metal ion is through the oxygen donors, whereas in basic solutions, there is an additional interaction of the copper(II) with the piperazinyl nitrogen donor.

A similar conclusion was drawn from the proton relaxation times (T_1) determined from the titration data in acidic and basic media in the Cu(II)–cfH system [122]. The authors claimed that it is plausible that more than one species is present in the solution at high pH values.

We can summarize that ^{1}H, ^{13}C and ^{19}F-NMR spectra have been used to study the behaviour of quinolones and their metal complexes in solution. The spectra of free quinolones were assigned and the chemical shift changes have confirmed the chelate bonding of metal ions to quinolone ring carbonyl and carboxylic oxygens. The changes in spectra also indicated that for quinolones bearing the piperazine ring at position 7, the terminal nitrogen of this ring system could be involved in the bonding to the metal in solution at higher pH values.

8. The mode of action of quinolones. Interactions of quinolones with DNA and the role of metal ions in these processes

Bacterial DNA topology is controlled by three enzymes, DNA gyrase introduces negative supercoiling; DNA topoisomerase I counters the action of gyrase to prevent the accumulation of excess supercoiling; and DNA topoisomerase IV plays a central role in the resolution of interlinked, replicated daughter chromosomes. Numerous classes of compounds have been demonstrated to interfere with both prokaryotic and eukaryotic enzymes. Among these, very active and widely prescribed drugs are currently being utilized for the treatment of bacterial infections and human cancers [123]. DNA gyrase and DNA topoisomerase IV are both sensitive to the 4-quinolone class of antibacterial compounds in vitro. The activity of quinolones is due to the inhibition of the supercoiling of DNA catalyzed by the enzyme DNA gyrase. Contradictory reports have appeared in the literature on the molecular details of drug–DNA and drug–enzyme interactions [124–143]. Shen and coauthors have proposed drug–DNA models which imply hydrogen-bond type interactions between the DNA unpaired bases and the quinolone, as well as a stacked dimerization of the drug [127–129]. Their results were also not quite consistent with the previously reported fact that nalidixic acid binds to single-stranded DNA only in the presence of an excess of copper ions [144]. The model has been modified and includes a possible interaction between the C-7 substituent and the quinolone pocket on the B subunit of DNA gyrase [145]. Palumbo et al. have stressed the role of magnesium in the quinolone–DNA interaction [146,147]. It was suggested that Mg(II) acts as a bridge between the quinolone and the phosphate group of the DNA, and that this complex is stabilized by stacking interactions between the condensed rings of the drug and the DNA bases in a single-stranded region or a distorted B-form in plasmid. In the subsequent study it was confirmed that DNA-affinity of the quinolone, modulated by Mg(II), plays an important role in poisoning the cleavable gyrase–DNA complex and, consequently, in eliciting antibacterial activity by this family of drugs. The results obtained with different 6-substituted compounds support the idea that position 6 of the drug, besides playing a pharmacokinetic role, is involved in recognition of the enzyme pocket. Llorente and coworkers [148] have proposed another model based on the intercalation of quinolone into the double helix of DNA. However, the results reported by Hurley et al. in their parallel study of quinobenzoxazines and nfH have shown that in the presence of Mg(II), only the former are able to form a stable intercalated complex with DNA [149]. It was later found that nfH, which can only play an external binding role, was able to modulate the photochemical reaction of the quinobenzoxazines on DNA [150].

Recently new studies have also been performed where an important role of metal ions in the mechanism of action of these drugs was again proposed. From a theoretical–experimental study on the structure and activity of certain quinolones and the interaction of their Cu(II) complexes on a DNA model, it was suggested that the intercalation of the quinolone complexed to a metal is an important step in these processes [151].

The conformational equilibria of DNA gyrase A (subunit of the enzyme) in the presence of Mg(II) and cfH were studied. It was proposed that the magnesium mediated quinolone binding to the enzyme might be involved in the mechanism of action of this family of drugs [152].

We can conclude that the mode of action of these drugs and related processes were extensively studied in the past. The topic is extremely important due to the fact that several quinolones are used in clinical practice. But obviously there are still several questions to be answered.

9. Bioactivity of quinolone–metal compounds

The complexes of iron(III)—$[Fe(nfH)_2(H_2O)_2]Cl_3 \cdot 6H_2O$ and zinc(II)–$[Zn(nfH)_2]Cl_2 \cdot 7H_2O$ were tested in vitro against the Gram negative microorganisms *E. coli* and *Bacillus dysenteria* bacteria [88]. The complexes showed stronger activity than nfH.

The biological activity of the Cu–phen–nal complex and its individual components was tested against *Entamoeba histolytica* (*HM 1*), *E. coli* and *Clostridium symbiosum* [153]. The complex showed the highest inhibitory activity on axenic and monoxenic amobease. *E. coli* showed high susceptibility to the Cu–phen–nal complex, whereas *C. symbiosum* did not. The authors concluded that Cu–phen–nal could have a potential use as an alternative drug in chemotherapy of some bacterial infections in disease caused by *E. histolytica*.

In contrast, the iron complex of nalH showed amoebical activity only at concentrations higher than those used with nalH [154].

The results of in vitro experiments demonstrated that under reductive conditions $[Cu(phen)(nal)]^+$ behaves as a powerful nuclease capable of degrading plasmid DNA [155]. These results support the hypothesis that the mechanism of action of quinolones could be mediated by a transition metal ion such as copper.

The complex $[Cu(cx)_2] \cdot 2H_2O$ was screened for activity against several bacteria (minimal inhibitory concentration (MIC) values) showing the same antimicrobial activity as the corresponding ligand [55]. The number of bacteria killed after 3 h of incubation with $[Cu(cx)_2] \cdot$

$2H_2O$ and $Na[Co(cx)_3]\cdot 10H_2O$ complexes was determined against *E. coli* (ATCC 25922) [63]. The copper compound presents a paradoxical effect—the diminution in the number of bacteria killed at high drug concentrations [156–159].

The compounds $(cfH_3)(cfH_2)[BiCl_6]\cdot 2H_2O$, $(kinoH_3)[CuCl_4]\cdot H_2O$ and $(erxH_3)[FeCl_4]Cl$ were tested against different microorganisms [66,71]. In the employed experimental procedure, the metal compounds showed the same antimicrobial activity against bacteria as the reference free quinolone drugs.

Two bismuth compounds, $(cfH_3)(cfH_2)[BiCl_6]\cdot 2H_2O$ and $(cfH_3)_2[Bi_2Cl_{10}]\cdot 4H_2O$, were tested against *Helicobacter pylori* [67]. The results show that the activity of both bismuth(III) compounds is comparable to that of cfH hydrochloride.

Two magnesium(II) complexes, $[Mg(FCl)_2]\cdot H_2O$ and $[Mg(cf)_2(H_2O)_2]\cdot 2H_2O$, were tested against various Gram positive and Gram negative microorganisms [160]. The results show that both magnesium complexes are significantly less active than the parent quinolone drugs. It was also found that the activity of quinolones is reduced when the solutions are titrated with magnesium ions.

It was also established that Cu(II) and Ni(II) are effective in induction of the cytotoxicity of some quinolones (nalH, oxoH) against leukemia cells in vitro, whereas Mg(II) was not effective [161]. The different effects of the metals on quinolone cytotoxicity can be explained by their different modes of interaction with quinolone. The authors claim that the transition metals can form DNA intercalated agents with quinolone, which can cause the cytotoxicity.

It was also found that the vanadium–cfH complex is promising with respect to its insulin-mimetic behaviour and concomitant low toxicity in the physiological concentration range [48].

Some of the isolated metal–quinolone complexes have been tested for activity against variety of microorganisms and some other biotests have also been performed. In most tests it was found that the activity of the complexes is comparable to free quinolones. In certain examples the activity was also increased (or lowered) but there is no evidence of any further clinical tests. It is expected that more such results are to be published in the future.

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