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A physico-chemical study of the interaction of ciprofloxacin and ofloxacin with polivalent cations

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Abstract

The reactions between ciprofloxacin and ofloxacin with different soluble salts of Al^{3+}, Mg^{2+}, Ca^{2+}, and Fe^{2+} in aqueous medium have been studied; the resulting products have been characterised using different analytical and spectroscopic techniques. Carboxylates are formed with ofloxacin, these salts being more soluble than the quinolone itself, due to its strong ionic character. However, ciprofloxacin does not form salts or non-absorbable complexes with metallic cations, although a crystalline material is formed. Experimental results using infrared and ^{1}H-NMR spectroscopies, mass spectrometry and X-ray diffraction indicate that the carboxylic group is unreacted. This new product, more stable than the corresponding metallic salts, does not contain any metallic atom/cation.

Key words: Quinolone; Ciprofloxacin; Ofloxacin; Metal-ion complexation; Antacid

1. Introduction

Several authors have reported on the interaction between quinolones and antacids or other drugs which include cations such as Al^{3+}, Mg^{2+}, Fe^{2+} or Ca^{2+}. A decrease in the amount of quinolone absorbed after its simultaneous oral administration with the above-mentioned cations has been found. The interaction is maximum for ciprofloxacin (Nix et al., 1989; Polk et al., 1989; Kara et al., 1991) or norfloxacin (Nix et al., 1990) and minimum, even though negligible, for ofloxacin (Flor et al., 1990; Martinez-Cabarga et al., 1991).

In spite of the large number of papers published regarding this subject, the mechanism of the interaction remains unclear. Several authors (Timmers and Sternglanz, 1978; Crumplin et al., 1980; Hoffken et al., 1988; Nix et al., 1989; Polk et al., 1989) suggest the formation of an insoluble, non-absorbable complex (coordination compound) between the quinolone and the corresponding cation. Others (Ross and Riley, 1990, 1992) suggest the formation of a 'micelar phase' as a consequence of changes in the net charge of the compound, and they attribute to this phenomenon the behaviour exhibited by quinolones in the presence of cations. These authors do not

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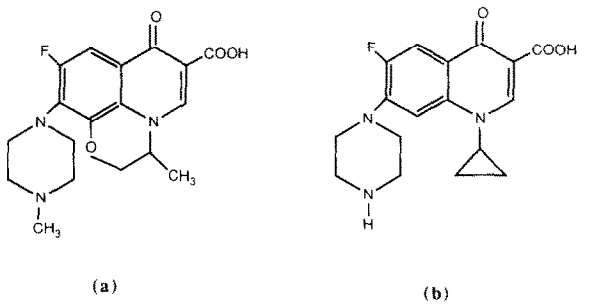


Fig. 1. Chemical structures of ofloxacin (a) and ciprofloxacin (b).

mention the formation of any non-absorbable complex as other authors do, and in no case has such a complex been identified.

The aim of the present study was to gain insight into the mechanism of this interaction from the chemical point of view, in order to understand the reactivity of quinolones with metallic cations, and hopefully to extend the conclusions to the reactivity in the gastrointestinal tract under similar experimental conditions.

Ciprofloxacin and ofloxacin (see structures in Fig. 1) were chosen as the quinolones to be studied, since they show important differences in absorption characteristics, in either the absence or presence of cations (Nix et al., 1989; Wolfson and Hooper, 1989; Flor et al., 1990).

2. Materials and methods

2.1. Materials

Aluminium nitrate and sulphate, iron(II) nitrate, magnesium nitrate and sulphate, and calcium carbonate and nitrate were supplied by Fluka (p.a.). Ciprofloxacin hydrochloride was obtained from Bayer and ofloxacin from Hoechst.

2.2. Instrumentation

Elemental chemical analysis for C, H, and N was performed in a Perkin-Elmer, model 2400 analyzer. The infrared spectra were recorded in a Perkin Elmer model FT-IR 1730 Fourier-transform spectrometer, coupled to a 3700 Data Station. Samples were analyzed using the KBr pellet technique, the pellets being obtained upon application of a pressure of 7 ton/cm^2. The crystalline structure was determined in an automatic, four circles CAD-4 diffractometer (Enraf Nonius), controlled by a DEC MicroVAXII computer, equipped with VAX/SDP (Frenz and Associayes, 1978) computing programmes, and SHELXS86 (Sheldrick, 1986) and ORTEP (Johnson, 1965) were also used. Data were collected at 293 K, using MoKα ($\lambda = 0.70930$ Å) radiation. The ^{1}H-NMR spectra were recorded on a Bruker WP200SY (200 MHz) instrument, using 1,4-dioxane as an internal standard and deuterated water as solvent. The mass spectra were obtained in a Kratos MS-250 apparatus, working at an ionization energy of 70 eV.

2.3. Preparation of the compounds

As our study was focused on the coordinative properties of these quinolones, although Al^{3+}, Mg^{2+}, and Ca^{2+} were initially chosen, we have also studied their reactivity with Fe^{2+}, a cation commonly present in drugs which will coordinate ligands more readily than the former cations. These cations are usually supplied to living organisms as hydroxides, which dissolve in the strong acidic medium existing in the gastric tract, however, we generally used soluble salts (e.g., nitrates and sulphates) in order to analyze their ability to undergo coordination by these molecules. The general method used for the synthesis was as follows: 0.16 mmol of ciprofloxacin hydrochloride (or ofloxacin) dissolved in 25 ml doubly distilled water were mixed with 0.08 mmol of hexahydrated magnesium nitrate previously dissolved in 5 ml water. After stirring for 15 min, the solution was stored in a vaccum dessicator on H_2SO_4 (conc.) until a solid was formed. Similar methods were used in all other cases, the molar ratio (quinolone/metal cation) being 2:1, and 3:1 for Al.

3. Results and discussion

Despite the structural analogy between these two molecules, their reactivity with these cations

was rather different. Therefore, whereas with ciprofloxacin the resulting solutions were almost colourless, solutions obtained with ofloxacin were deep yellow (Al^{3+}), light brown (Mg^{2+}, Ca^{2+}) and even dark brown (Fe^{2+}). The colour of these solutions strengthened as the concentration was increased, however, in all cases those prepared with ciprofloxacin were much lighter than compared to those with ofloxacin. Such a colour change would generally be associated with the formation of soluble coordination compounds between these molecules and the metallic cations. The initial pH of the solution was 4.7–5.2 for ofloxacin solutions and 6.0–6.3 for ciprofloxacin solutions (due to the use of the former quinolone as a hydrochloride), and in both series of studies the pH was adjusted to 5.0.

After standing in vacuo for 3 or 4 days, colourless needle-like crystals were formed in ciprofloxacin solutions. However, no crystal was formed in the case of ofloxacin solutions; a solid was isolated only when the solution was completely dried. The general aspect of the crystals was rather similar, irrespective of the metallic cation.

Elemental chemical analysis of the crystals obtained from the ciprofloxacin containing solutions indicated that no metal existed in the crystals. However, the compositions of the crystals did not correspond to ciprofloxacin hydrochloride, but to the compositions ciprofloxacin · HNO_3 or ciprofloxacin · H_2SO_4, i.e., it appeared that HCl existing in the starting reagent had been substituted by the acid forming the starting metal salt. These results were reproducible (within experimental error) for different preparations. In contrast, the solids isolated after drying solutions containing ofloxacin and metallic salts obviously incorporated the corresponding metal cation in their composition. Moreover, chemical analyses of different preparations were slightly different, and the C, H and N contents were always lower than those corresponding to the stoichiometric composition $M(ofloxacin)_2$ (or $Al(ofloxacin)_3$). These results indicated that these solids were not pure, and might still have incorporated a portion of unreacted, starting reagents, together with reaction by-products.

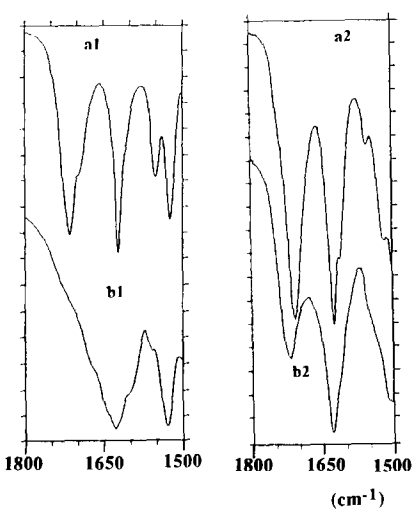


Fig. 2. IR spectra (1800–1500 cm^{-1}): (a1) ofloxacin; (b1) product isolated after reaction with different metal cations; (a2) ciprofloxacin, (b2) product isolated after reaction with different metal cations.

The FT-IR spectra of the solids were almost coincident, irrespective of the metal cation. Representative spectra are included in Fig. 2. Only the 1800–1500 cm^{-1} range is shown, since changes in the spectra due to the formation of coordination compounds were mostly expected in this region. Hence, profile a1 corresponds to the initial ofloxacin; the strong, sharp band at 1713 cm^{-1} is due to $\nu C=O$ of the free carboxylic group, while the $\nu C=O$ of the ketonic group is recorded at 1622 cm^{-1}. After reaction with the metal salts, the $\nu C=O$ (-COOH) band vanished from the spectra of the solid residue (b1) and instead a broad band at 1625 cm^{-1}, corresponding to the $\nu_{as}(COO)$ of carboxylate, is recorded; this band also includes that of the ketonic group. Therefore, it could be concluded that reaction with the metal cation took place through ionization of the carboxylic group to carboxylate, but without participation (or only weak participation) as a coordinating moiety of the ketone group.

The spectra of the crystals isolated from the ciprofloxacin-containing solutions were rather different. Consequently, the $\nu C=O$ (-COOH) band at 1708 cm^{-1} in the original ciprofloxacin shifted approx. 20 cm^{-1} to higher wavenumbers in the spectra of the crystals; this means that the

carboxylic group remained unreacted. Such a shift has been ascribed to a decrease in the number and/or intensity of the hydrogen bonds existing in this molecule. All other bands remained unchanged. In addition, bands ascribed to nitrate or sulphate were also recorded, thus further confirming incorporation of these compounds into the crystals. Summarizing, it appears that ciprofloxacin did not react with these metal cations, but that the changes originated from different dispositions of the molecules, due to the substitution of HCl by HNO_3 or H_2SO_4, respectively.

A first conclusion can be drawn from the results described so far. While ofloxacin reacts with the metallic cations in the expected manner (however, the pure compounds were not isolated), the behaviour shown by ciprofloxacin in the presence of the metallic cations was completely different, and therefore we focused our further studies on the nature of these compounds. Moreover, isolation of single crystals allowed determination of their precise composition and structure by X-ray diffraction. Such a study was carried out on the nitrate derivatives, of which the purity and crystallinity were greater than those of the corresponding sulphates.

Electron density maps were corrected using the polarization and Lorentzian factors, in order to localize all atoms, excluding H. Only the parameters of one molecule (of the two that seemed to constitute the unit cell) were determined due to the uncertainity originating from the similarities of all constitutuent atoms (C, N, O and F). Interpretation of the electron density map differences or refiniment of the localized atoms was not completely satisfactory. The unrefined coordinates of the atoms in the ciprofloxacin molecule are given in Table 1; the bond lengths and bond angles are listed in Table 2. The crystallographic data are summarized in Table 3, and the structure of the molecule (indicating the numbering code used) is shown in Fig. 3.

As demonstrated by the above results, no metallic atom existed in the solids isolated after mixing solutions containing ciprofloxacin and metallic salts; i.e., no coordination compound was formed. The existence of one other molecule in the unit cell (although not completely identified, it appeared to be different from that shown in Fig. 3) indicates some sort of molecular association. On the other hand, the C–O distances are not equivalent, and C_1–O_2 is shorter than C_1–O_1, indicating C = O and C–O moieties, respectively, the O_1 atom thus probably being bonded to the hydrogen atom, constituting a free carboxylic group, -COOH. The other interatomic distances show values expected for the bonds existing in similar species.

Table 1
Atomic coordinates of the ciprofloxacin molecule

Atom	x	y	z
F	−0.026	0.752	0.104
O(1)	−0.179(3)	0.214(3)	0.110(6)
O(2)	0.014(5)	0.072(4)	0.142(8)
O(3)	−0.172(3)	0.408(3)	0.101(6)
N(1)	0.229(4)	0.306(4)	0.165(7)
N(2)	0.257(3)	0.678(2)	0.152(5)
N(3)	0.403(4)	0.827(3)	0.095(7)
C(1)	−0.048(4)	0.178(3)	0.140(6)
C(2)	0.024(5)	0.261(5)	0.138(9)
C(3)	−0.045(7)	0.376(6)	0.13 (1)
C(4)	0.033(4)	0.456(4)	0.137(8)
C(5)	−0.028(4)	0.563(4)	0.119(8)
C(6)	0.046(5)	0.646(4)	0.109(8)
C(7)	0.180(4)	0.605(4)	0.149(8)
C(8)	0.242(5)	0.493(5)	0.161(9)
C(9)	0.166(8)	0.421(6)	0.16 (1)
C(10)	0.153(5)	0.232(5)	0.163(9)
C(11)	0.367(8)	0.267(7)	0.20 (1)
C(12)	0.445(8)	0.157(6)	0.13 (1)
C(13)	0.457(8)	0.272(7)	0.08 (1)
C(14)	0.387(6)	0.657(6)	0.18 (1)
C(15)	0.446(6)	0.716(5)	0.03 (1)
C(16)	0.263(4)	0.862(3)	0.053(6)
C(17)	0.20 (1)	0.798(9)	0.17 (2)

In order to determine whether the structure shown by these crystals was maintained in solution (both the IR spectra and the crystal structure were determined in the solid state), the ^{1}H-NMR of these compounds were also recorded. Chemical shifts for pure ciprofloxacin and for the crystals isolated after mixing with the different metal salts are given in Table 4. Firstly, the same number of signals was recorded for pure ciprofloxacin as for the corresponding crystals. Secondly, only minor shifts were observed in some cases, which

Table 2
Bond lengths and bond angles in the ciprofloxacin molecule

Bond distances (pm)			
F–C(6)	132.6	C(2)–C(10)	131.6
O(1)–C(1)	133.9	C(3)–C(4)	147.5
O(2)–C(1)	131.4	C(4)–C(5)	133.2
O(3)–C(3)	129.7	C(4)–C(9)	136.0
N(1)–C(9)	141.3	C(5)–C(6)	148.3
N(1)–C(10)	141.5	C(6)–C(7)	139.0
N(1)–C(11)	141.5	C(7)–C(8)	137.2
N(2)–C(7)	149.7	C(8)–C(9)	137.2
N(2)–C(14)	132.8	C(11)–C(12)	146.5
N(2)–C(17)	136.2	C(11)–C(13)	127.2
N(3)–C(15)	140.8	C(12)–C(13)	153.6
N(3)–C(16)	144.1	C(14)–C(15)	156.5
C(1)–C(2)	146.7	C(16)–C(17)	148.8
C(2)–C(3)	141.8		
Bond angles (°)			
C(9)–N(1)–C(10)	119.0	F–C(6)–C(5)	115.7
C(9)–N(1)–C(11)	120.0	F–C(6)–C(7)	128.5
C(10)–N(1)–C(11)	120.7	C(5)–C(6)–C(7)	114.3
C(7)–N(2)–C(14)	121.6	N(2)–C(7)–C(6)	115.8
C(7)–N(2)–C(17)	121.6	N(2)–C(7)–C(8)	121.5
C(14)–N(2)–C(17)	115.2	C(6)–C(7)–C(8)	122.3
C(15)–N(3)–C(16)	106.3	C(7)–C(8)–C(9)	118.9
O(1)–C(1)–O(2)	120.9	N(1)–C(9)–C(4)	118.6
O(1)–C(1)–C(2)	117.2	N(1)–C(9)–C(8)	118.8
O(2)–C(1)–C(2)	121.2	C(4)–C(9)–C(8)	122.5
C(1)–C(2)–C(3)	120.0	N(1)–C(10)–C(2)	124.7
C(1)–C(2)–C(10)	120.9	N(1)–C(11)–C(12)	123.2
C(3)–C(2)–C(10)	118.8	N(1)–C(11)–C(13)	130.1
O(3)–C(3)–C(2)	121.0	C(12)–C(11)–C(13)	67.9
O(3)–C(3)–C(4)	121.3	C(11)–C(12)–C(13)	50.1
C(2)–C(3)–C(4)	117.6	C(11)–C(13)–C(12)	62.0
C(3)–C(4)–C(5)	119.6	N(2)–C(14)–C(15)	109.4
C(3)–C(4)–C(9)	121.1	N(3)–C(15)–C(14)	101.1
C(5)–C(4)–C(9)	119.3	N(3)–C(16)–C(17)	110.5
C(4)–C(5)–C(6)	121.9	N(2)–C(17)–C(16)	110.9

Table 3
Crystallographic data for ciprofloxacin

Formula	$C_{17}H_{18}FN_3O_3$
Molecular weight	331.35
Crystal system	triclinic
Space group	P1
a (pm)	1066.6
b (pm)	1259.0
c (pm)	663.2
α (°)	92.76
β (°)	93.86
γ (°)	73.27
Volume (pm^3)	850.5×10^6
Z	2
ρ_x ($g\ cm^{-3}$)	1.294
μ (cm^{-1})	0.915
$F(000)$	174

could be due to the change in the counteranion (nitrate or sulphate instead of chloride) or to a different association (rearrangement) of the quinolone molecules.

Therefore, the ^{1}H-NMR results indicated that the molecular network was maintained in these crystals isolated from solutions containing ciprofloxacin and the metal salts; no functional group seemed to be lost or modified.

Similar conclusions were reached from the mass spectrometric analysis carried out. The MS patterns recorded for pure ciprofloxacin and for the crystals obtained are shown in Fig. 4. The peak at $m/e = 368$ corresponds to ciprofloxacin · HCl, while the intense peak at $m/e = 331$ corresponds to the molecular peak of ciprofloxacin. This peak is also recorded in the patterns of the crystals (Fig. 4). Lack of detection of the peaks corresponding to ciprofloxacin · HNO_3 or ciprofloxacin · H_2SO_4 may be due to the facile removal of the acid molecules under the vacuum conditions in the spectrometer chamber. The origin of other major peaks at lower m/e values could be due to the residues existing after removal of the carboxylic group ($m/e = 287$) and of the cyclopropyl group ($m/e = 245$).

The results obtained so far indicate that ofloxacin and ciprofloxacin behave very differ-

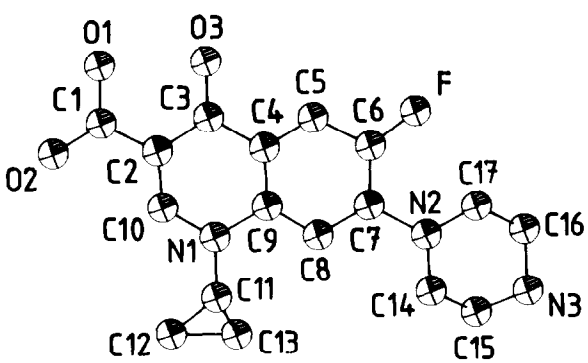


Fig. 3. Structure of the ciprofloxacin molecule, according to the X-ray diffraction results.

ently when reacting with metal cations, despite the analogy existing between their structures. So, reaction of ofloxacin with metal cations follows the general pattern expected, leading to the formation of salts or coordination compounds. The lack of reactivity of ciprofloxacin could be due to some sort of molecular association existing between these molecules, thus making less favourable its rupture and reaction with the metal cations. Such an intermolecular association appears to exist both in the solid state (as shown by the FT-IR and X-ray diffraction results) and in solution (as shown by the ^{1}H-NMR results). If so, this association would explain the weaker absorption of ciprofloxacin when administered to patients together with any of the above-mentioned cations (Al^{3+}, Mg^{2+}, Fe^{2+} and Ca^{2+}), although it remains unclear why this effect seems to be evident only in the presence of metal cations. It could be claimed that the metal cations play the role of a template, very well known in other areas of coordination chemistry, that permits the synthesis of large molecules from residues previously coordinated to a given metal cation.

In contrast, ofloxacin maintains its absorption characteristics when administered to patients together with cations. In other words, the different behavior of these quinolones, established from a physicochemical point of view, is clearly shown regarding pharmacokinetic properties. This fact again confirms the strong relationship between the physicochemical properties of drugs and their pharmacokinetic behaviour.

We also wish to stress that the conditions used in this study are far removed from those existing in vivo, especially with regard to the amounts of reagents used. Hence, while in vivo the quinolone/metal ion ratio is of the order of 1:500, we used ratios of 2:1 and 3:1, in order to prepare defined compounds, taking into account the formal oxidation state of the cations studied and the monoprotic character of these quinolones. The use of such large contents of metal cation could give rise to adsorption processes, as already reported to exist between aluminium hydroxide

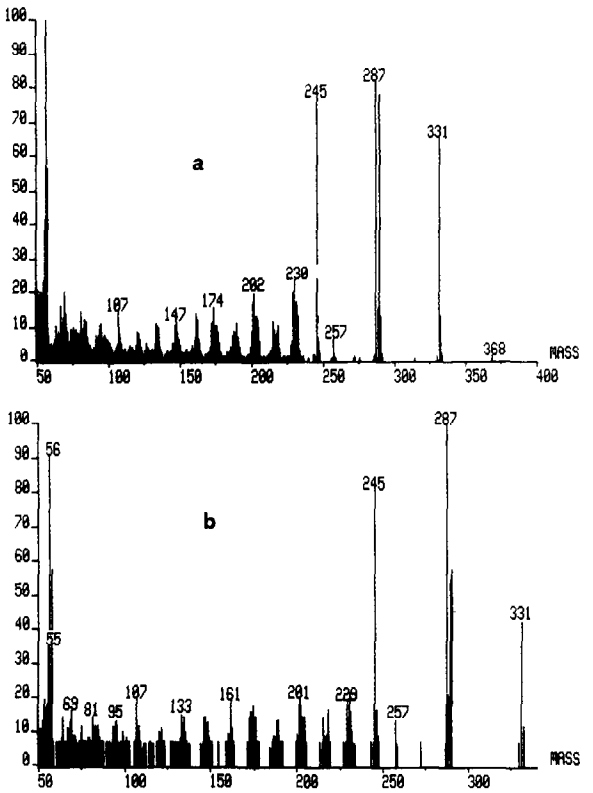
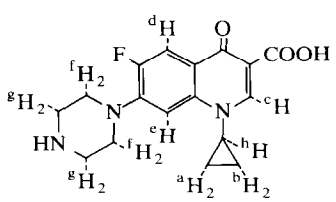


Fig. 4. Mass spectra: (a) ciprofloxacin, (b) product isolated after reaction with different metal cations.

Table 4
Chemical shifts in the ^{1}H-NMR spectra of ciprofloxacin (Cipro) and the products isolated after reaction with different metal cations

	Cipro	$Mg(NO_3)_2$ Cipro	$Al(NO_3)_3$ Cipro	$Al_2(SO_4)_3$ Cipro
[a] H	1.31	1.31	1.20	1.19
[b] H	1.05	1.09	1.02	0.98
[c] H	8.40	8.59	8.51	8.57
[d] H	7.31	7.48	7.52	7.55
[e] H	7.18	7.42	7.39	7.40
[f] H	3.41	3.41	3.25	3.25
[g] H	3.49	3.49	3.38	3.37
[h] H	–	3.61	3.53	3.52



and alizarine or morine (Burriel et al., 1989), that would account for the different absorption properties of the quinolones in the presence of cations, due to their high specificity.

In any case, we should conclude that formation of defined compounds (salts or coordination compounds) between ciprofloxacin and metal cations, that some authors have claimed to explain the weak absorption of the quinolone, does not take place.

Acknowledgments

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