

Synthesis, Spectroscopic Characterization and Antibacterial Activities of Co(II) Complex of Ofloxacin Drug Mixed with Ascorbic Acid as secondary ligand

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as [doi: https://doi.org/10.32350/BSR.0303.01](https://doi.org/10.32350/BSR.0303.01)

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ABSTRACT

Ofloxacin is a quinolone antibiotic that is considered an efficient antibacterial drug with a broad spectrum of activity against anaerobic and aerobic bacteria and has strong antibacterial activity *in vitro* against many bacteria species by inhibiting their DNA-gyrase. In this study, the synthesis, physicochemical and spectroscopic characterization of Cobalt (II) metal complex with ofloxacin as primary ligand and ascorbic acid as the secondary ligand have been carried out. The complex was prepared by reflux method for four hours in methanol. The complex, with the molecular formula [Co(OfI)(Asc)], was characterized by its color, solubility, melting point, FTIR, UV/Visible, ¹H NMR, and ¹³C NMR spectroscopy. The color and the melting point suggest that complexation occurred. The Fourier Transform Infrared data for both the primary ligand (OfI) and the secondary ligand (Asc) acted as tridentate ligands. OfI coordinated to the Co(II) metal ion via the two carbonyl oxygen atoms and the oxygen atom of the hydroxyl group, whereas Asc coordinated to the metal through the carbonyl and enolic C-2 and C-3 hydroxyl groups. The electronic data suggests octahedral geometry for the complex. The ligands and the novel Co (II) complex were tested for *in vitro* antibacterial activity against gram-negative and gram-positive bacterial species using the filter paper disc agar diffusion method. Significant antibacterial activities were observed for the complex compared to the ligands. This research will aid in the development of more potent drugs that are resistant to organisms.

KEYWORDS: Mixed ligand, Ofloxacin, Ascorbic acid, cobalt (II), antibacterial activity.

INTRODUCTION

Ofloxacin is a quinolone antibiotic that is considered an efficient antibacterial drug with a broad spectrum of activity against anaerobic and aerobic bacteria and has strong antibacterial activity *in vitro* against many bacteria species by inhibiting their DNA-gyrase. [1, 2, 3]. Ofloxacin is an antibiotic used to treat bacterial infections of the skin, Chlamydia and/or gonorrhoea, prostate urinary tract infections as well as a pelvic inflammatory disease.

Researchers have recently been attempting to develop novel chemical compounds to prevent bacterial resistance to an antibiotic, which is one of the most serious issues confronting the medical community [4]. And one of such discoveries is the complexation of drugs with metal ions which improves the pharmaceutical profiles of these drugs. Studies on the development of mixed-ligand complexes are also important in the fields of biological and environmental chemistry [5] [6]. The antibacterial activities of Co(II) and Ni(II) mixed complexes of ofloxacin with 1,10-phenanthroline (phen)/2,2'-bipyridine (bipy) have been reported [4]. A large number of mixed-ligand complexes from primary ligands such as gemifloxacin, flumequine, lomefloxacin, norfloxacin, oxolinic acid, and enrofloxacin with some nitrogen donor heterocyclic ligand, such as 1,10-phenanthroline (Phen), 2,2'-bipyridine (Bipy), and amino acid such as glycine (Gly) acting as secondary ligand have been synthesized with Zn(II), Zr(IV), La(III), Ce(III), Ce(IV), Th(IV), Sn(II), and U(VI) metal ions have been reported [7 – 9]. The antibiotic activities of the mixed ligand complexes were carried out *in vitro*. The mixed ligand complexes displayed higher biological effectiveness than the parent ligands against the bacterial species tested [7 – 9].

A lot of metal complexes of ofloxacin have been reported in combination with other ligands, no report has been reported in the literature for the Co(II) mixed ligand complex of ofloxacin and ascorbic acid. Therefore, it was thought of interest to carry out synthesis, physicochemical and spectroscopic characterization of mixed ligand complex of cobalt(II) with ofloxacin and ascorbic acid. The study also includes antibacterial activities of

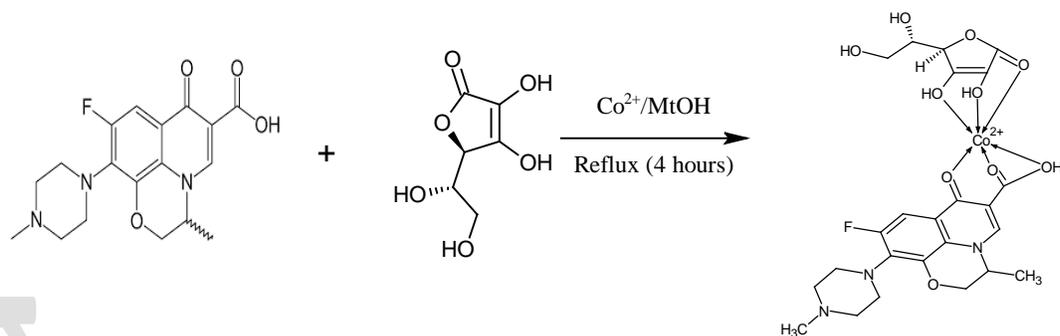
the synthesized complex and the ligands against some bacterial species. The results of these investigations are reported in this article.

METHODOLOGY

The reagents and chemicals utilized for this study were analytical grade and were used without further purification. Cobalt(II) sulfate received from Loba Chemie LTD. Mumbai 400005, India. The ligands, Ofloxacin, and ascorbic acid were received from Mancare pharmaceuticals PVT. LTD. India. Stuart melting point apparatus was used to determine the melting points of the ligands and the Co(II) mixed ligand complex. KBr pellets were used to record the vibration spectra of the ligand and complex on a Shimadzu FTIR 8400S model Spectrophotometer in the range 4000–400 cm^{-1} . The electronic spectra of the ligand and Co(II) mixed ligand complex were recorded on a UV/Visible spectrophotometer (UV-2500PC series). Proton and Carbon -13 NMR spectra were recorded on a JEOL JNM-EX 270 spectrometer. Deuterated dimethylsulfoxides (DMSO – d₆) were used as solvents. The solubilities of the ofloxacin, ascorbic acid, and the Co(II) mixed ligand complex were determined in different solvents such as distilled water, methanol, DMF, DMSO, chloroform acetone, and petroleum ether.

Synthesis of Co(II) mixed ligand complex

The synthesis of the Co(II) mixed ligand complex was performed by the method described by Al-Saif *et al.*, [5] with slight modification. The complex was prepared by dissolving 10.84 g (30 mmol) of ofloxacin in 20 cm^3 methanol. An aqueous solution of 8.34 g (30 mmol) $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was added and stirred vigorously with a magnetic stirrer. To the solution, 5.28 g (30 mmol) ascorbic acid was added with continuous stirring and the solution was refluxed for 4 hours at 70 – 80 °C. The brown solid product was filtered and allowed to dry under anhydrous CaCl_2 vacuum desiccators.



Scheme 1: Equation of reaction for the synthesis of [Co(OfI)(Asc)]

Antibacterial studies

The synthesized cobalt complex, ofloxacin, and ascorbic acid were tested for antibacterial activity against Gram-positive and Gram-negative bacteria species namely; *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Candida albicans* clinically isolated bacterial strains and comparisons were made. The bacteriological growth medium was nutrient agar. The bacterial activities in the presence of both the parent drug and the Co(II) mixed ligand complex were determined by the filter paper disc agar diffusion method [10]. The antibacterial activity of the drugs was estimated by measuring the size of the zone of inhibition on seeded nutrient agar, around the wells.

RESULTS AND DISCUSSION

Physical properties of ofloxacin, ascorbic acid, and their mixed ligand complex are presented in Table 1. The mixed complex is colored. This means that visible light excites an electron from a level occupied by an electron in a complex's molecular orbital to an empty level, resulting in visible spectrum absorption. The colors are either due to d - d electron transitions or charge transfer from the ligands to the Co^{2+} ion [11]. The Co(II) complex is a non-hygroscopic and thermally stable solid with a high melting point indicating metal - ligand bonds [12 - 17]. The Solubility data of ofloxacin, ascorbic acid, and their Co(II) mixed ligand complex are shown in Table 2. The mixed complex was found to be insoluble in acetone, chloroform, Petroleum ether, and distilled water but soluble in DMF and DMSO.

IR Spectrum of the Ligands and Co(II) Complex

The infrared spectra of ofloxacin and ascorbic acid were compared with that of the mixed ligand complex (Table 3). The stretching frequencies at 3260 cm^{-1} , 3520 cm^{-1} and 3360 cm^{-1} in the free ascorbic acid due to

OH stretching vibration, was shifted to 3335.03 cm^{-1} in Co(II) complex. The shift supported that coordination has occurred through this point. The OH stretching vibration of the ofloxacin ligand at 3500 cm^{-1} was shifted in the complex which also suggested coordination at the hydroxyl functional group. The vibration frequency at 1674 cm^{-1} in ascorbic acid and 1633.33 cm^{-1} in the free ofloxacin, were assigned to C=O stretching vibrations, these bands have been shifted in the mixed complex to 1700.31 cm^{-1} . This also suggested coordination through C=O. The peaks at 430.14 cm^{-1} which could not be traced in the spectrum of the free ligands have been tentatively assigned to Co-O vibrations [18].

Electronic spectra

The electronic spectral data of ofloxacin, ascorbic acid, and their mixed ligand complex are presented in Table 4. The absorption band at 254 nm in ascorbic acid and the bands at 220, 300, and 375 nm in the ofloxacin ligands were assigned to intra-ligand charge transfer transition (ILCT), due to chromophoric groups in the ligands. In the mixed ligand complex, the bands were observed to have undergone a bathochromic shift due to complexation [19]. The electronic transition of $[\text{Co}(\text{OfI})(\text{Asc})]_x$ mixed complex showed three bands at 768.50, 320.50, and 313.00 nm corresponding to ${}^4\text{T}_1 \rightarrow {}^4\text{T}_2$, ${}^4\text{T}_1 \rightarrow {}^4\text{A}_2$, and LMCT respectively. The octahedral geometry for this compound was proposed by these transitions [29]. Consequently, the ${}^4\text{T}_1 \rightarrow {}^4\text{T}_2$ and ${}^4\text{T}_1 \rightarrow {}^4\text{A}_2$ transitions were used to calculate the ligand field parameter (Dq), the Racah parameter (B), and the Nephelauxetic effect (β) using the T-S diagrams.

For the ligand field parameter Dq, the calculated value is 11760.84 cm^{-1} (ν_1) (Table 5). Thus, for the Co(II) complex, the interelectronic repulsion parameter, or Racah parameter B, was calculated and found to be 420.03 cm^{-1} . The nephelauxetic ratio $\beta = B/B^0 = 0.3750$ (37.5%) indicated appreciable covalent character in the complex [20 – 22]. The decrease is a general observation that electron repulsions in the complex are weaker than in free atoms and ions. The occupied molecular orbitals delocalize over the ligands and away from the metal, resulting in the weakening. The delocalization increased the average separation of the electrons and consequently reduced their mutual repulsion [20][23].

NMR Spectra

The proton NMR spectra assignment of the ofloxacin and ascorbic acid ligands were compared with their mixed complex to ascertain the points of coordination (Table 6). The chemical shift of the carboxylic OH group in ofloxacin was observed at 14.76 ppm while the OH groups of ascorbic acid were observed at 10.95 and 11.02 ppm respectively. In the spectrum of [Co(OfI)(Asc)], the carboxylic OH group of ofloxacin shifted to 11.45 ppm while the OH group of ascorbic acid shifted to 13.02 and 13.15 ppm respectively. These shifts suggested the participation of carboxylic OH, and OH of ascorbic acid in coordination with the formation of M-O bond which corresponds to the data collected from the infrared spectrum [30] [31].

The ^{13}C NMR spectrum of ofloxacin and ascorbic acid ligands was compared with their complex (Table 7). The chemical shift value for the C=O carboxylic acid of ofloxacin appeared at 165.60 ppm while the C=O of ketone appeared at 175.63 ppm. The carbonyl group in the ascorbic acid was assigned a chemical shift value of 173.96 ppm. In the ^{13}C NMR spectrum of [Co(OfI)(Asc)]_x, the C=O carboxylic acid of ofloxacin shifted to 183.67 ppm and the C=O of ketone group shifted to 196.02 ppm while the carbonyl group in ascorbic acid shifted to 184.53 ppm. These shifts suggested the involvement of C=O of ascorbic acid) in complexation. The proposed structure of the complex is shown in Figure 1.

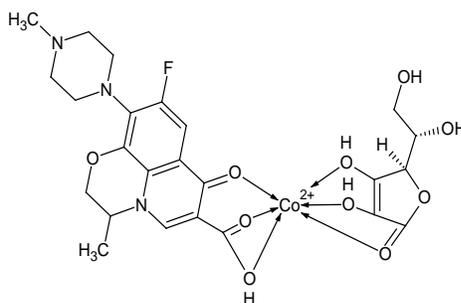


Figure 2: Proposed structure of [Co(OfI)(Asc)]_x

Antibacterial studies

The antibacterial studies of ofloxacin, ascorbic acid, and their complex were tested against five bacteria species; namely *S. aureus*, *E. coli*, *B. subtilis*, *P. aeruginosa*, and *C. Albicans*. The zone of inhibitions is presented in Table 8. A comparison of the antibacterial activity of the Co(II) complex against the five bacteria species elicited significantly better ($P < 0.05$) antibacterial than the free ligands. It was observed that metal

chelation has affected significantly the antibacterial behavior of the ligands [24] [25]. Overtone's Concept and Chelation Theory explained why ligand activity increased upon complexation. Chelation, according to this theory, lowers a metal atom's polarity by sharing a portion of its positive charge with donor groups and possible π -electron delocalization across the entire ring. As a result, the complex becomes more lipophilic, making it easier for it to pass through the lipid layer of the cell membrane. Metal-binding sites in microorganism enzymes are blocked by the complex. Hence, the complex disturbs the metabolism pathways in the cell, leading to the extinction of microorganisms [26][27]. The result showed that ascorbic acid possessed no activity against all the five bacteria species. The results are in agreement with the earlier report of their non-activity [28]

Minimum Inhibitory Concentration of ofloxacin and Co(II) mixed ligand complex are presented in Table 9. In ofloxacin and $[\text{Co}(\text{OfI})(\text{Asc})]_x$, the result showed that there was no growth of microbes inoculated at 0.1 $\mu\text{g}/\text{ml}$. This suggested that the minimum inhibition concentration was 0.1 $\mu\text{g}/\text{ml}$.

CONCLUSION

The mixed ligand metal complex of Co(II) has been synthesized, characterized by FT-IR, UV-Visible, and NMR spectral studies. The results of these investigations supported the suggested structure of the complex which was found to have octahedral geometry. The mixed ligand complex is a high melting point colored complex. The color of the mixed complex was attributed to charge transfer from the ligand to the metal ion or d - d electron transitions. The ligand and mixed ligand complex was tested for antibacterial efficacy against the microorganisms; *S. aureus*, *E. coli*, *B. subtilis*, *P. aeruginosa*, and *C. Albicans*. The Co(II) mixed ligand complex of ofloxacin-ascorbic acid showed higher antibacterial activity compared with the free ligands. It was observed that the antibacterial behavior of the Co(II) mixed ligand complex was appreciably higher ($P < 0.05$) than the free ligands against the tested species.

Conflict of Interest

The authors declare no conflict of interest.

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Table 1: physical properties of ofloxacin, ascorbic acid and their mixed ligand complex

Ligand/ Complex	Colour	Melting Point (°C)	Yield (%)
Ofloxacin	White	250–257	-
Ascorbic Acid	White	190	-
[Co(OfI)(Asc)] _x	Brown	> 300	19.54

OfI = Ofloxacin, Asc = Ascorbic acid

Table 2: Solubility data of ofloxacin, ascorbic acid and their mixed ligand complex

Ligand/ Complexes	Distilled water	DMSO	DMF	Methanol	Acetone	Chloroform	Petroleum Ether
Ofloxacin	SS	S	S	S	NS	SS	NS
Ascorbic Acid	S	S	S	SS	NS	NS	NS
[Co(OfI)(Asc)] _x	SS	S	S	NS	NS	NS	NS

NB:S = Soluble, SS = Slightly Soluble, NS = Not soluble, OfI = Ofloxacin, Asc = Ascorbic acid

Table 3: Selected IR Spectral data of ofloxacin, ascorbic acid and their mixed ligand complex

Ligand/ Complexes	ν OH (cm ⁻¹)	ν C-H (cm ⁻¹)	ν C=O (cm ⁻¹)	ν M-O (cm ⁻¹)
Ofloxacin	3500.00	2933.33	1633.33	-
Ascorbic Acid	3260.00 3520.00 3360.00	2922.10	1674	-

[Co(OfI)(Asc)] _x	3335.03	2937.68	1700.31	430.14
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OfI = Ofloxacin, Asc = Ascorbic acid

Table 4: Electronic spectral data of ofloxacin, ascorbic acid and their mixed ligand complex

Ligand/ Complex	Wavelength (nm)	Energy (cm ⁻¹)	Assignment
Ofloxacin	220.00	45454.55	ILCT ($\pi \rightarrow \pi^*$)
	300.00	33333.33	ILCT ($\pi \rightarrow \pi^*$)
	325.00	30769.23	ILCT ($\pi \rightarrow \pi^*$)
Ascorbic Acid	254	39370	ILCT ($\pi \rightarrow \pi^*$)
[Co(OfI)(Asc)] _x	768.50	13020.83	${}^4T_1(F) \rightarrow {}^4T_2(F)$
	320.50	31201.25	${}^4T_1(F) \rightarrow {}^4A_2(F)$
	313.00	31948.88	LMCT

OfI = Ofloxacin, Asc = Ascorbic acid

Table 5: Crystal field splitting energy (Dq), Racah parameter(B) and Nephelauxetic effects (β) of the mixed ligand complex

Complex	Dq (cm ⁻¹)	B (cm ⁻¹)	β	Dq/B	ν_1/B	ν_2/ν_1
[Co(OfI)(Asc)] _x	11760.84	420.03	0.3750	28	31	2.39

OfI = Ofloxacin, Asc = Ascorbic acid

Table 6: ¹H NMR spectral data of ofloxacin, ascorbic acid and its complex

Compound	Hydroxyl protons (ppm)	Methyl, methylene protons (ppm)	Aromatic protons (ppm)
Ofloxacin	14.76	1.12, 2.30, 2.50, 3.19, 3.30, 3.82	7.92, 8.65

Ascorbic	2.84, 10.95, 11.02	3.64, 3.78,	-
[Co(OfI)(Asc)] _x	11.45, 13.02, 13.15	1.15, 2.32, 2.53, 2.90, 3.17	7.69, 6.88, 6.11,

OfI = Ofloxacin, Asc = Ascorbic acid

Table 7: ¹³C NMR spectral data of ofloxacin, ascorbic acid and their mixed ligand complex

Compound	C=O (ppm)	COOH (ppm)	Ar Carbons (ppm)	CH ₃ and CH ₂ (ppm)
Ofloxacin	175.63	165.60	106.40, 126.30, 127.30, 132.60, 143.10, 148.00, 158.40, 158.60	19.36, 47.30, 49.70, 54.95, 57.95, 71.00
Ascorbic	173.96	-	-	77.13, 69.91, 63.25, 156.25, 118.83
[Co(OfI)(Asc)] _x	196.02, 184.53	183.67	106.42, 114.68, 126.01, 127.31, 132.61, 143.11, 148.02	19.34, 47.32, 54.98, 57.44, 63.29, 70.31, 71.01, 71.03, 76.61,

OfI = Ofloxacin, Asc = Ascorbic acid

Table 8: The zones of inhibition (mm) of ofloxacin, ascorbic acid and their mixed ligand complexes against some selected microorganism

	<i>S. aureus</i>	<i>E. Coli</i>	<i>Bacillus subtilis</i>	<i>Pseudominas aeruginosa</i>	<i>Candida albicans.</i>
Ascorbic acid	NA	NA	NA	NA	NA
Ofloxacin	36.03 ^b ± 0.05	32.37 ^b ± 0.55	37.27 ^b ± 1.42	36.13 ^b ± 0.15	40.67 ^b ± 0.55
[Co(OfI)(Asc)] _x	43.33 ^a ± 0.57	40.33 ^a ± 0.58	45.01 ^a ± 0.01	43.03 ^a ± 0.06	41.07 ^a ± 0.11

Values are means ± standard deviation of triplicate determination.

a-b means bearing different superscripts in the same column are significantly different (P < 0.05) while means with the same superscript shows no significant difference (P > 0.05).

OfI = Ofloxacin, Asc = Ascorbic acid

Table 9: The minimum inhibitory concentrations of ofloxacin and its mixed ligand complexes against some selected microorganisms

	Conc. $\mu\text{g/ml}$	<i>S. aureus</i>	<i>E. Coli</i>	<i>Bacillus subtilis.</i>	<i>Pseudominas aeruginosa</i>	<i>Candida albicans</i>
Ofloxacin	0.1	-	-	-	-	-
	0.05	-	-	-	+	-
	0.025	+	+	+	+	+
[Co(OfI)(Asc)]	0.1	-	-	-	-	-
x	0.05	-	-	+	-	-
	0.025	+	+	+	+	+

+ = Growth observed in medium; - = Absence of growth in medium.
OfI = Ofloxacin, Asc = Ascorbic acid