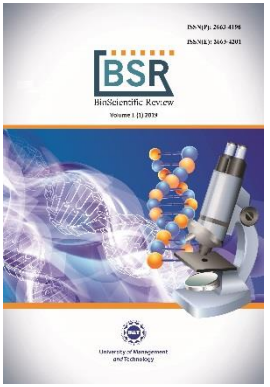

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Synthesis, Spectroscopic Characterization and Antibacterial Activities of Co(II) Complex of Ofloxacin Drug Mixed with Ascorbic Acid as Secondary Ligand

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Abstract

Ofloxacin is a quinolone antibiotic. It is considered an efficient antibacterial drug with a broad spectrum of activity against anaerobic and aerobic bacteria. Moreover, it shows 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 the primary ligand and ascorbic acid as the secondary ligand were achieved. The complex was prepared by using the reflux method for four hours in methanol. The complex, with the molecular formula [Co(Ofl)(Asc)], was characterized by its color, solubility, melting point, FTIR, UV/Visible, ^{1}H NMR, and ^{13}C NMR spectroscopy. The color and the melting point suggested that complexation occurred. The Fourier Transform Infrared data for both the primary ligand (Ofl) and the secondary ligand (Asc) acted as tridentate ligands. Ofl coordinated with the Co(II) metal ion via the two carbonyl oxygen atoms and the oxygen atom of the hydroxyl group, whereas Asc coordinated with the metal through the carbonyl and enolic C-2 and C-3 hydroxyl groups. Electronic data suggested 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 against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis, and Candida albicans* for the complex as compared to the ligands. This research will aid in the development of more potent drugs that are resistant to organisms.

1. Introduction

Ofloxacin is a quinolone antibiotic that is considered an efficient antibacterial drug with a broad spectrum of activity against anaerobic and aerobic bacteria. It also shows 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 gonorrhea, prostate urinary tract infections, and the pelvic inflammatory disease (PID).

Researchers have recently attempted to develop novel chemical compounds to prevent bacterial resistance to antibiotics, which is among the most serious issues confronting the medical community [4]. A newly discovered method to develop such compounds 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 field 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 composed of primary ligands such as gemifloxacin, flumequine, lomefloxacin, norfloxacin, oxolinic acid, and enrofloxacin, along with a nitrogen donor heterocyclic ligand such as 1,10-phenanthroline (Phen), 2, 2'-bipyridine (Bipy), and amino acids such as glycine (Gly) acting as a secondary ligand 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 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. However, no study was found in the literature for the Co(II) mixed ligand complex of ofloxacin and ascorbic acid. Therefore, it was thought of as the subject of interest to carry out the synthesis, physicochemical and spectroscopic characterization of the mixed ligand complex of cobalt(II) with ofloxacin and ascorbic acid. The current study also covers the antibacterial activities of the synthesized complex and the ligands against some bacterial species. The results of these investigations are reported in this article.

2. Methodology

The reagents and chemicals utilized for this study were analytical grade and used without further purification. Cobalt(II) sulfate was 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 both the ligand and the 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

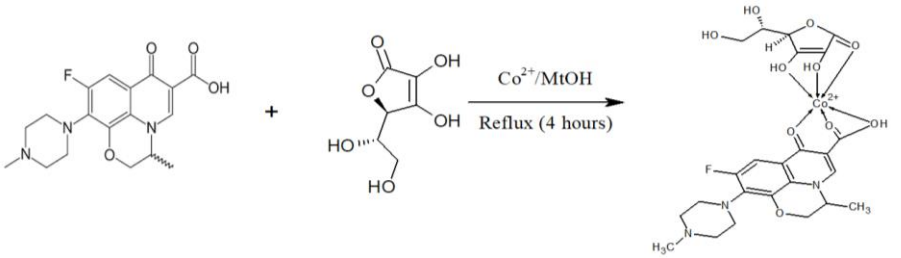

Figure 1. Equation of reaction for the synthesis of [Co(Ofl)(Asc)]

spectrometer. Deuterated dimethylsulfoxides (DMSO – d6) were used as solvents. The solubilities of 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.

2.1. 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 4.64 g (30 mmol) $CoSO_4$ was added and stirred vigorously with a magnetic stirrer. To the solution, 5.28 g (30 mmol) of ascorbic acid was added with continuous stirring and the solution was refluxed for 4 hours at 70 – 80ºC. The brown solid product was filtered and allowed to dry under anhydrous $CaCl_2$ vacuum desiccators (Figure1).

2.2. 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 were prepared and comparisons were made. The bacteriological growth medium was nutrient agar. Bacterial activities in the presence of both the parent drug and the Co(II) mixed ligand complex were determined using 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 of seeded nutrient agar around the wells.

3. Results

Physical properties of ofloxacin, ascorbic acid, and their mixed ligand complex are presented in Table 1. The solubility data of ofloxacin, ascorbic acid, and their Co(II) mixed ligand complex are presented 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.

3.1. 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). Stretching frequencies at 3260 cm^{-1}, 3520 cm^{-1} and 3360 cm^{-1} in the free ascorbic acid due to OH stretching vibration were shifted to 3335.03 cm^{-1} in Co(II) complex. The OH

Table 1. Physical properties of ofloxacin, ascorbic acid and their mixed ligand complex

Ligand / Complex	Colour	Melting Point (^{O}C)	Yield (%)
Ofloxacin	White	250–257	-
Ascobic Acid	White	190	-
$[Co(Ofl)(Asc)]_x$	Brown	> 300	19.54

Ofl = Ofloxacin, Asc = Ascorbic acid

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

Ligand / Complex	Distilled water	DMSO	DMF	Methanol	Acetone	Chloroform	Petroleum Ether
Ofloxacin	SS	S	S	S	NS	SS	NS
Ascobic Acid	S	S	S	SS	NS	NS	NS
$[Co(Ofl)(Asc)]_x$	SS	S	S	NS	NS	NS	NS

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

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

Ligand / Complex	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	-
$[Co(Ofl)(Asc)]_x$	3335.03	2937.68	1700.31	430.14

Ofl = Ofloxacin, Asc = Ascorbic acid

stretching vibration of the ofloxacin ligand at 3500 cm^{-1} was also shifted in the complex. Vibration frequencies 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 were shifted in the mixed complex to 1700.31 cm^{-1}. The peaks at 430.14 cm^{-1}, which could not be traced in the spectrum of the free ligands, were tentatively assigned to Co-O vibrations.

3.2. Electronic Spectra

The electronic spectral data of ofloxacin, ascorbic acid, and their mixed ligand complex are presented in Table 4. Absorption bands were observed at 254 nm in ascorbic acid and at 220, 300, and 375 nm in the ofloxacin ligands. In the mixed ligand complex, the bands were observed to have undergone a bathochromic shift. Electronic transition of $[Co(Ofl)(Asc)]_x$ mixed complex showed three bands at 768.50, 320.50, and 313.00 nm, corresponding to $^4T_1 \rightarrow {}^4T_2$, $^4T_1 \rightarrow {}^4A_2$ and LMCT, respectively. The $^4T_1 \rightarrow {}^4T_2$ and $^4T_1 \rightarrow {}^4A_2$ transitions were used to calculate the ligand field parameter (Dq), the Racah parameter (B), and the Nephelauxetic effect (β) using the T-S diagrams.

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 ($\pi \rightarrow \pi^*$)
$[Co(Ofl)(Asc)]_x$	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

Ofl = 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})	B	Dq/B	ν_1/B	ν_2/ν_1
$[Co(Ofl)(Asc)]_x$	11760.84	420.03	0.3750	28	31	2.39

Ofl = Ofloxacin, Asc = Ascorbic acid

For the ligand field parameter Dq, the calculated value is 11760.84 cm^{-1} (v1) (Table 5). Thus, for the Co(II) complex, interelectronic repulsion parameter or Racah parameter B was calculated to be 420.03 cm^{-1}. The nephelauxetic ratio was $\beta = B/B^O = 0.3750$ (37.5%).

3.3. NMR Spectra

The proton NMR spectra assignments of the ofloxacin and ascorbic acid ligands were compared with their mixed complex to ascertain the points of coordination (Table 6). 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(Ofl)(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. 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 ascorbic acid was assigned a chemical shift value of 173.96 ppm. In the ^{13}C NMR spectrum of $[Co(Ofl)(Asc)]_x$, 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 2.

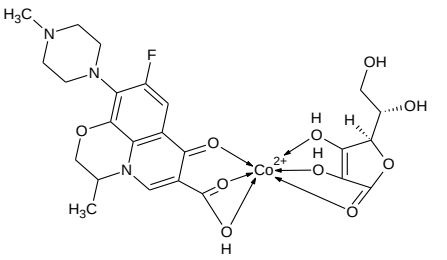

Figure 2. Proposed structure of [Co(Ofl) (Asc)]$_x$

Table 6. ^{1}H 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,	-
$[Co(Ofl)(Asc)]_x$	11.45, 13.02, 13.15	1.15, 2.32, 2.53, 2.90, 3.17	7.69, 6.88, 6.11,

Ofl = Ofloxacin, Asc = Ascorbic acid

Table 7. ^{13}C 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
$[Co(Ofl)(Asc)]_x$	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,

Ofl = Ofloxacin, Asc = Ascorbic acid

Table 8. Zones of inhibition (mm) of ofloxacin, ascorbic acid and their mixed ligand complex against selected microorganism

	S. aureus	*E. Coli*	*Bacillus subtilis*	*Pseudominas aeruginosa*	*Candida albicans.*
Ascorbic acid	NA	NA	NA	NA	NA
Ofloxacin	$36.03^{b} \pm 0.05$	$32.37^{b} \pm 0.55$	$37.27^{b} \pm 1.42$	$36.13^{b} \pm 0.15$	$40.67^{b} \pm 0.55$
$[Co(Ofl)(Asc)]_x$	$43.33^{a} \pm 0.57$	$40.33^{a} \pm 0.58$	$45.01^{a} \pm 0.01$	$43.03^{a} \pm 0.06$	$41.07^{a} \pm 0.11$

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 the same superscript shows no significant difference ($P > 0.05$). Ofl = Ofloxacin, Asc = Ascorbic acid

Table 9. Minimum inhibitory concentrations of ofloxacin and its mixed ligand complex against selected microorganisms

	Conc. *µg/ml*	*S. aureus*	*E. Coli*	*Bacillus subtilis.*	*Pseudominas aeruginosa*	*Candida albicans*
Ofloxacin	0.1	-	-	-	-	-
	0.05	-	-	-	+	-
	0.025	+	+	+	+	+
$[Co(Ofl)(Asc)]_x$	0.1	-	-	-	-	-
	0.05	-	-	+	-	-
	0.025	+	+	+	+	+

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

3.4. Antibacterial Studies

The antibacterial effects 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 zones of inhibition are presented in Table 8. A comparison of the antibacterial activity of the Co(II) complex against the five mentioned bacterial species elicited significantly better ($P < 0.05$) antibacterial results than the free ligands. It was observed that metal chelation affected significantly the antibacterial behavior of the ligands. Overtone's Concept and Chelation Theory explained why ligand activity increased upon complexation. The results showed that ascorbic acid performed no activity against all the five bacteria species. The minimum inhibitory concentration of ofloxacin and Co(II) mixed ligand complex are presented in Table 9. In ofloxacin and $[Co(Ofl)(Asc)]_x$, the results showed that there was no growth of microbes inoculated at 0.1 µg/ml. This suggested that the minimum inhibition concentration was 0.1 µg/ml.

4. Discussion

The coloured complex suggests that visible light excites an electron from the level occupied by it 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 stretching frequencies at 3260 cm^{-1}, 3520 cm^{-1} and 3360 cm^{-1} in the free ascorbic acid due to OH stretching vibration were shifted to 3335.03 cm^{-1} in Co(II) complex. The shift purported that coordination has occurred through this point. The OH stretching vibration of the ofloxacin ligand at 3500 cm^{-1} shifted in the complex, which also suggested coordination in the hydroxyl functional group. Vibration frequency at 1674 cm^{-1} in ascorbic acid and 1633.33 cm^{-1} in the free ofloxacin was assigned to C=O stretching vibrations, these bands shifted in the mixed complex to 1700.31 cm^{-1}. This shift 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].

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 the presence of chromophoric groups in the ligands. In the mixed ligand complex, the bands were observed to have undergone a bathochromic shift due to complexation [19]. Electronic transition of $[Co(Ofl)(Asc)]_x$ mixed complex showed three bands at 768.50, 320.50, and 313.00 nm, corresponding to $^4T_1 \rightarrow {}^4T_2$, $^4T_1 \rightarrow {}^4A_2$, and LMCT, respectively. The octahedral geometry for this compound was proposed by these transitions [20]. Consequently, the $^4T_1 \rightarrow {}^4T_2$ and $^4T_1 \rightarrow {}^4A_2$ 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} (v1). The nephelauxetic ratio $\beta = B/B^O = 0.3750$ (37.5%) indicates appreciable covalent character in the complex [21, 22, 23]. The decrease corroborates the 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. Delocalization increased the average separation of the electrons and consequently reduced their mutual repulsion [21, 24].

The chemical shift of the carboxylic OH group in ofloxacin in NMR spectra 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(Ofl)(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 [25, 26].

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, when the ^{13}C NMR spectra of ofloxacin and ascorbic acid ligands were compared. The carbonyl group in ascorbic acid was assigned a chemical shift value of 173.96 ppm. In the ^{13}C NMR spectrum of $[Co(Ofl)(Asc)]_x$, 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 suggest the involvement of C=O of ascorbic acid in complexation. The antibacterial studies of ofloxacin, ascorbic acid, and their complex tested against five bacteria species namely *S. aureus, E. coli, B. subtilis, P. aeruginosa, and C. Albicans* showed that metal chelation affected significantly the antibacterial behavior of the ligands [27, 28]. 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 causes 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 [29, 30]. The results showed that ascorbic acid showed no activity against all the five mentioned bacteria species. These results concur with the earlier report of their non-activity [31].

5. Conclusion

The mixed ligand metal complex of Co(II) was synthesized and characterized by FT-IR, UV-Visible, and NMR spectral studies. Their results 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 their 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 as 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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