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Synthesis, spectral characterization and antimicrobial activity of some M(II) complexes with Ciprofloxacin

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Abstract
Ciprofloxacin, CFL is a drug that belongs to the second generation of fluoroquinolone antibiotics with a wide range of effects on Gram-positive and Gram-negative bacteria. The aim of this work was to investigate the interaction of CFL as ligand with divalent biological cations (Mn^{2+}, Ni^{2+} and Co^{2+}) in approximate physiological conditions. Synthesized complexes were characterized using FTIR and stereo-microscopy. Antimicrobial screening was performed on bacterial strains of *Escherichia coli*, *Salmonella* Enteritidis, *Enterococcus faecalis* and *Staphylococcus aureus*.
The results of FTIR spectroscopy showed that the M(II) complexes with CFL were formed through the oxygen donors of the carboxyl and carbonyl group of the ligand. Stereo-microscopic characterization revealed the difference in color and size of crystals of the ligand and metal complexes. Antimicrobial screening has shown that CFL and complexes have almost similar antimicrobial activity against investigated bacterial strains.

1. INTRODUCTION

Ciprofloxacin (CFL) is a synthetic antibacterial fluoroquinolone related to nalidixic acid having a fluorine atom and piperazine ring at the positions 6 and 7 of quinolone-3-carboxylic acid (Nagalapalli and YagaBheem, 2014). It is a broad-spectrum antibiotic, sensitive to gram-negative and more effective against gram-positive bacteria, including *Staphylococcus aureus, Streptococcus pneumoniae* and *Enterococcus faecalis* (Ali et al., 2010). It is used intravenously in the form of lactate salts (Al-Omar, 2005). The structure of ciprofloxacin is shown in Figure 1. Ciprofloxacin, first introduced in 1987, is one of the most widely used fluoroquinolones now (Choi et al., 2013). According to the World Health Organization, CFL is one of the most frequently prescribed antimicrobial drugs (Correia et al., 2017). CFL impedes the replication and transcription of bacterial DNA, leading to an increase in oxidative stress and death of bacterial cells (Masadeh et al, 2016).

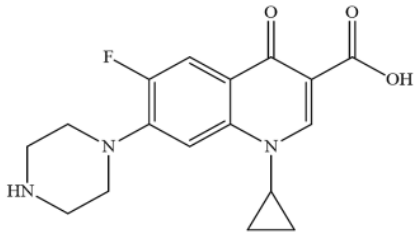


Figure 1. Structure of CFL

CFL is the most effective quinolone against *Pseudomonas aeruginosa* (Lebel, 1988). It is applied in the treatment of infections of the lower respiratory tract, skin, soft tissue, sexually transmitted diseases, and urinary tract infections (Fàbrega et al, 2009). CFL is the first fluoroquinolone, which has shown significant activity outside the urinary tract. Quinolones realize their effect by converting gyrases and

topoisomerases IV into toxic enzymes that fragment bacterial chromosome (Aldred et al., 2014). CFL, like other quinolones, inhibits DNA gyrase, but its bacteriological effects are not completely reversible. Therefore, unlike many other quinolones, ciprofloxacin may have multiple lethal effects (Sanders, 1988). CFL has been observed that it can induce inhibition of growth, depending on time and dose, and apoptosis of various carcinoma, osteosarcoma and others. (Sharma et al., 2010). It induces S-phase arrest and augment apoptotic effects of cisplatin in human pancreatic cancer cells (Yadav et al., 2015). This drug is partially metabolized in the liver by modifying the piperazinyl group to at least four metabolites. These metabolites, identified as desethyleneciprofloxacin, sulfo-ciprofloxacin, oxo-ciprofloxacin and N-acetyl ciprofloxacin, exhibit weaker microbiological activity than the starting form of the drug, but similar or stronger activity relative to some other quinolones (Al-Omar, 2005). Serum concentrations of metabolite are less than 10% of CFL levels, even in reduced renal function (Bergan et al, 1989). Various compounds are introduced into the body by food or drugs, which may potentially interact with metals and thereby affecting their biological effect (Cipurković et al., 2017). Metal complexes stay a significant resource for creating chemical diversity in the fields of biological, pharmaceutical and medicinal chemistry as antitumor and antimicrobial agents (Ljubijankić et al., 2018). Analysis of the interaction of biological M(II) ions with O, N and S-donor atoms of ligands, often used in the treatment of a wide spectrum of diseases, is important for pharmacokinetics, monitoring distribution, excretion, drug efficacy and monitoring adverse effects (Cipurković et al., 2017). Input and transport of manganese (Mn) ions are important for all organisms. Mn plays an important role in the metabolism of lipids, proteins and carbohydrates (Porcheron et al., 2013). Its absorption in the gastrointestinal tract takes place in a divalent and tetravalent form (Barceloux D. G. and Barceloux D., 1999). Cobalt (Co) is a core element of vitamin B12 (cyanocobalamin), which is essential for the prevention of anemia in humans (Huwait et al., 2015). Co is thought to enhance erythropoietin expression by replacing the essential Fe^{2+} in the HIF-α dioxygenases (Jelkmann, 2011). Co is found in human organisms in the amounts of 1 to 2 mg (Cezarnek et al, 2015). Nickel (Ni) is functional microelement for the activity of urease enzymes (). Ni facilitates the absorption of iron (Fe^{3+}) in the intestines, and it is also cofactor in metalloenzymes and metalloproteins (Samal and Mishra, 2011). Ni is necessary for the biosynthesis of hydrogenase, carbon monoxide dehydrogenase. It is found in a large number of bacteria (Can et al, 2014). Nickel can influence human health through infectious diseases caused by nickel-dependent bacteria (Zambelli et al, 2013). This bio element and its compounds are well known as carcinogens (Oller, 2002). However, the identity of these compounds, which increase the risk of cancer, is still unclear (Barceloux D.G. and Barceloux D., 1999). The aim of this paper is to examine the interaction of some biological essential transition metal ions with CFL in model systems in approximately physiological conditions.

2. MATERIAL AND METHODS

2.1. Synthesis of complex

A mixture of ethanol and water, in a ratio of 50/50 (*v/v*), was used as a solvent for ligand and M(II) salts. Metals (M) and ligand (L) were mixed in a 1:2 molar ratio (n/n). The solutions of the appropriate metal (10 mL) and ligand (10 mL) were mixed in a glass and stirred on a magnetic stirrer, without heating. pH value of solutions were adjusted with 1M NaOH. The optimum pH value for all model systems was 7.3. Prepared solutions were mixed in magnetic stirrer for half an hour and then left to stand in a darkened area for two weeks in order to precipitate complexes. The resulting products were filtered through a blue strip filter paper and then dried at room temperature. Characterizations of obtained products were performed as earlier described (Cipurković et al., 2017).

2.2. FTIR characterization

In order to determine structure of the complex, samples were recorded on Nicolet iS10 FT-IR spectrophotometer - Thermo Fisher Scientific.

2.3. Morphological characterization

Before morphological characterization, solid complexes were treated with DMSO. Microscopic analysis were performed in order to compare color, texture, ligand particle size. Shots were performed on the binocular microscope, the Leica DM 2500P mark. Samples were recorded in polarized light, with and without crossed Nicole (XPL and PPL).

2.4. *In vitro* antimicrobial activity

Antimicrobial activity was investigated by diffusion method on reference bacterial strains (from ATCC collection) from gram positive (*Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 51299) and gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Salmonella* Enteritidis ATCC 13076). From the bacterial strains of overnight cultures, suspensions of 0.5 McFarland turbidity were prepared (density 107-108 CFU/mL, depending on soy). The strains were then placed on the surface of the nutrient substrate-Mueller-Hinton agar (MH), dispersed in sterile Petri dishes. Substrate thickness was 4 mm. In the agar sterile drill-shaped holes were made ("wells"), into which 50 μL of CFL and different M(II) complexes solutions in concentration of 1 mg/ml were added. After the plates were left at room temperature for 15 minutes, the substance was diffused into agar, incubated at 37^0C/24 h. After the incubation period, the size of the inhibitory zone was measured and the sensitivity of the microorganisms was expressed as follows: if the zone for inhibition of microorganism growth was greater than 20 mm, it was labeled with three pluses (+++), representing the highest sensitivity of the microorganisms. If the inhibitory zone ranged from 16 to 20 mm, it was marked with two pluses (++). Very weak sensitivity is marked with a plus (+) if the inhibitory zone is 10-15 mm in diameter. For the inhibitory zone less than 10 mm or if absent, the minus (-) has been used (Pirvu et al., 2014).

3. RESULTS AND DISCUSSION

3.1. Structure of the complex

The proposed structure of CFL complexes with metal ions (Mn^{2+}, Co^{2+} or Ni^{2+}) is shown in Figure 2.

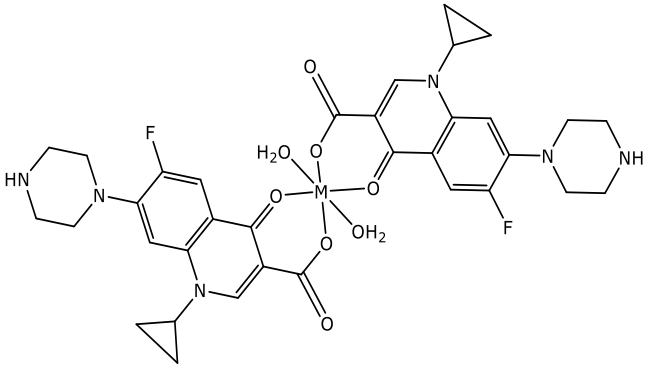


Figure 2. Structure of ML_2 complex (M = Co^{2+}, Ni^{2+}, Mn^{2+}, L = CFL)

Taking into account the molar ratio of the reactants, as well as the obtained FTIR spectra for the synthesized complexes, we conclude that CFL coordinates metal ions as bidentate O donor ligand in molar ratio of CFL:M = 2:1. One oxygen atom of ligand carbonyl group and one oxygen atom from the deprotonated -COOH group are involved in chemical bonds with M(II) ions, forming the octahedral complex. Similar research was performed by other scientists (Chohan et al., 2005). According to their results, two molecules of water are also coordinated on metal ion. The obtained complexes differ in color from the parent ligand. The complex $Co(CFL)_2(H_2O)_2$ is light pink, $Mn(CFL)_2(H_2O)_2$ is light brown and $Ni(CFL)_2(H_2O)_2$ is light green.

3.2. FTIR characterization

Figure 3 shows FTIR spectra of ligands and obtained complexes.

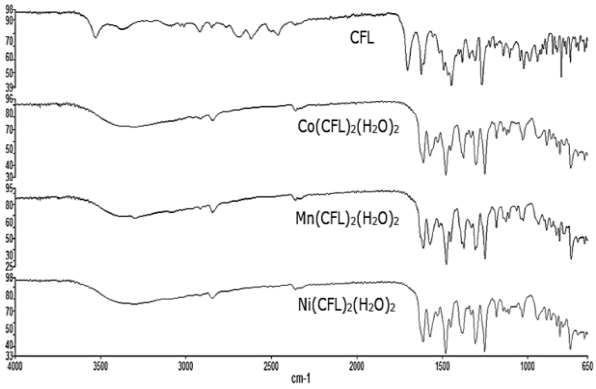


Figure 3. FTIR spectra of CFL and metal complexes

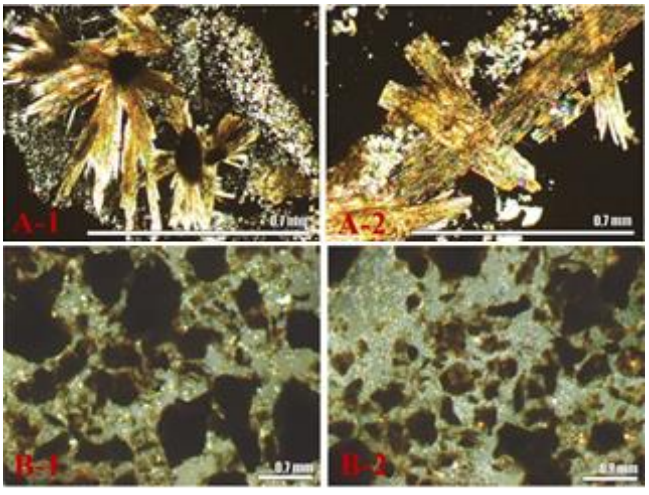
Characteristic bands for ligand and synthesized complexes are presented in Table 1. There are two very strong absorption bands in the spectrum of CFL at 1705,6 and 1624 cm^{-1} assigned to *v*COOH and *v*C=O stretching vibrations. On comparison of these IR frequencies, these bands completely vanished in the spectra of the metal complexes. Instead, the strong absorption bands positioned at 1571–1572,3 and 1372–1375,6 cm^{-1} indicating that *v*COOH group emerged as two absorption bands $\nu_{asymmet}$ COO and ν_{symmet}COO and its coordination with the metal atoms. Similarly, the band at 1624 cm^{-1} due to the C=O moiety in the spectrum of the drug ligand disappeared and instead a new band at 10–15 cm^{-1} lower frequency (~1610 cm^{-1}) appeared indicating involvement of the carbonyl group in coordination. On the basis of these changes we can propose that the drug ligand, CFL is acting as bidentate O-donor.

Table 1. Selected FTIR spectral vibrations of CFL and its metal complexes (cm^{-1})

Group	CFL	$M(CFL)_2(H_2O)_2$		
		Ni^{2+}	Mn^{2+}	Co^{2+}
O-H (COOH)	3529	-	-	-
N-H	3373	3342	3347	3356
C=O	1624	1613	1611	1611
C=O (COOH)	1705	-	-	-
C-O	1022	1030	1026	1026
C-F	1267	1252	1250	1249
C-N	1307	1307	1307	1303

3.3. Morphological characterization

The morphologies of CFL crystals and its complexes with Co^{2+}, Ni^{2+} and Mn^{2+} are presented in Figure 4. Crystals of CFL (Figures A-1 and A-2) are of small (below 1 mm) and medium size (1-10 mm). Interferential colors are alive and of first order. These crystals appear radially airy coming from a common center (Figure A-1) or have prismatic forms (Figure A-2). The similarity of the crystalline forms can be seen between CFL and the complex $Ni(CFL)_2(H_2O)_2$ (Figures D-1 and D-2) with radially airy structure of crystals. Crystals of radial forms of Ni(II) complex are significantly smaller then CFL crystals (almost 40%). These crystals have prismatic forms, which size is about 0.1 mm (Figure D-2). Unlike the crystals in Figure A-2, these are significantly smaller and do not have lammels. Complexes of Co(II) (Figure B-1 and B-2) and Mn(II) (Figures C-1 and C-2) do not develope crystals after treatment with DMSO.



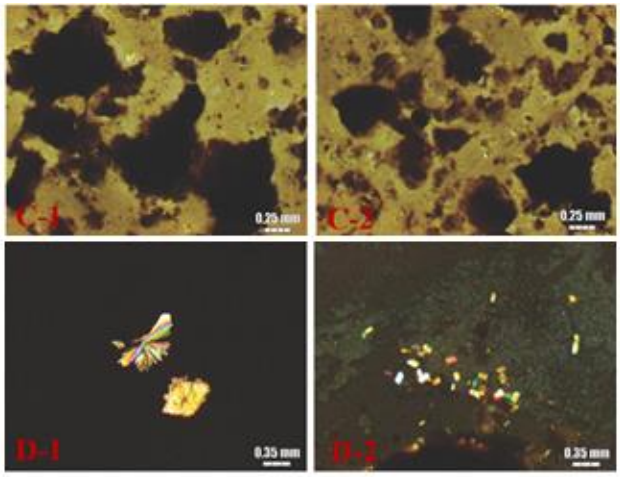


Figure 4. Morphology of (A) CFL crystals, (B) Co(CFL)2(H2O)2 crystals, (C) Mn(CFL)2(H2O)2 crystals and (D) Ni(CFL)2(H2O)2 crystals

The samples of these crystals are very small (so-called microliths, sizes below 0.02 mm) with irregular (polygonal) forms. Due to the coagulation, polarized light could not pass through these samples, so black or dark brown aggregates, thickness over 0.03 mm, were obtained.

3.4. Antimicrobial activity *in vitro*

Results of the *in vitro* antimicrobial activity of synthesized complexes against selected bacterial strains are shown in Table 2.

Table 2. Antimicrobial activities of CFL and its complexes $M(CFL)_2(H_2O)_2$

Ligand/Complexes	Inhibition zone [mm]
Escherichia coli (ATCC 25922)	
CFL	40
$Mn(CFL)_2(H_2O)_2$	35
$Co(CFL)_2(H_2O)_2$	42
$Ni(CFL)_2(H_2O)_2$	36
Staphylococcus aureus (ATCC 25923)	
CFL	35
$Mn(CFL)_2(H_2O)_2$	35
$Co(CFL)_2(H_2O)_2$	35
$Ni(CFL)_2(H_2O)_2$	33
*Salmonella*Enteritidis (ATCC 13076)	
CFL	39
$Mn(CFL)_2(H_2O)_2$	38
$Co(CFL)_2(H_2O)_2$	36
$Ni(CFL)_2(H_2O)_2$	35
Enterococcus faecalis (ATCC 51299)	
CFL	27
$Mn(CFL)_2(H_2O)_2$	30
$Co(CFL)_2(H_2O)_2$	28
$Ni(CFL)_2(H_2O)_2$	25

In the case of *E. coli*, the inhibitory activity ranges from 35 mm (for $Mn(CFL)_2(H_2O)_2$) to 42 mm (for

$Co(CFL)_2(H_2O)_2$). Inhibition zone for *S. aureus* is almost the same, while for *Salmonella* Enteritidis was observed less antimicrobial activity of the complex than for antibiotic (CFL >$Mn(CFL)_2(H_2O)_2$ > $Co(CFL)_2(H_2O)_2$ > $Ni(CFL)_2(H_2O)_2$). In the case of *E. faecalis,* antimicrobial activity of complexes is greater than to the parent ligand (except for $Ni(CFL)_2(H_2O)_2$). In comparison with the results obtained by Chohan and al. (2005), different values of antimicrobial activities for bacterial strains of *E. coli* and *S. aureus* are observed. The inhibition zones that they published for these strains are less than the results we obtained. In both cases, analyzes were performed for CFL and its complexes concentrations of 1 mg/mL, but different sample volumes were used (they used 100 µL, which is twice more than the volumes we used). However, there is a difference in the larger inhibitory zones of some M(II) complexes compared with parent antibiotic. In the results published by Chohan and al, all complexes have greater antimicrobial activities than CFL. For many chelates of quinolones obtained in solid state, an equal, lower or superior biological activity was observed compared to that of parent drugs. The consequence of interaction with metal ions on the biological activity of quinolones was approached as a negative phenomenon, bringing to reduction in the antimicrobial activity of quinolones in the presence of metal ions. The reduction of CFL activity by metal cations could be explained as the formation of an inactive chelate or decreased permeation of the antibiotic into bacterial cells (Ma et al., 1997). From the other side, increased biological activity of metal chelates can be explained by the concept of chelation theory and cell permeability. Delocalization of pi electrons over the whole chelate ring increases the lipophilic nature of the central ion which enhances the passage of complex through the lipid membranes and the penetration in cells (Tumer et al., 1999; Imran et al., 2007; Patel et al., 2007).

4. CONCLUSION

Metal ions are known to affect the action of many drugs structure quinolones. In this paper we have studied interaction of some biometal ions with CFL in water/ethanol solution. According to the obtained FTIR spectra, it is confirmed that CFL as ligand co-ordinate as bidentate ligand through one of the oxygen atoms of the deprotonated carboxyl group and the ring carbonyl atom. Synthesized complexes are not structurally different from similar complexes that were published by other scientists earlier. It is proposed that Co^{2+}, Ni^{2+} and Mn^{2+}ions form with CFL the octahedral complex in approximately physiological conditions. Based on morphological data, it has been determined that forms of CFL crystals are radially airborne and prismatic. Morphological similarity of Ni(II) complex with CFL was observed, unlike the complexes of Mn(II) and Co(II) with CFL. The antimicrobial activities of $M(CFL)_2(H_2O)_2$ were performed on four bacterial strains and compared with the activity of parent ligand. Antimicrobial activities were decreased for complexes with regard to the parent ligand, except in the case of $Co(CFL)_2(H_2O)_2$ complex activity on *E. coli* and $Mn(CFL)_2(H_2O)_2$ and $Co(CFL)_2(H_2O)_2$ complexes activities on *E. faecalis*.

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