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Electrochemistry, DFT Calculations, and Antioxidant Capability of Cobalt Cefazolin Complex

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Abstract: The synthesis, characterization, electrochemistry, theoretical investigation and antioxidant capability of cobalt cefazolin [Co(Cefaz)Cl] complex are reported here. Electrochemical characterization of the complex was carried out by using cyclic voltammetry (CV) and Linear Sweep Voltammetry (LSV) methods. Two irreversible oxidative responses and one quasi-reversible reduction couple were observed for the complex on the voltammograms. The ground state energies of the molecules have been estimated using B3LYP functional with LanL2DZ basis set based on time dependent density functional theory (TD-DFT). According to the theoretical calculation, [Co(Cefaz)Cl] complex can be expected to exhibit greater reactivity from Cefazolin (Cefaz) ligand, and the higher antioxidant properties were observed for the complex. The experimental results are in good agreement with the theoretical calculations.

Keywords: Cefazolin complex, electrochemistry, DFT calculation, antioxidant capability.

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INTRODUCTION

Cephalosporins are one group of beta-lactam antibiotics (1). The basis of the cephalosporin molecule forms from beta-lactam and dihydrothiazine rings attached to the cefem group. The cefem group leads to the complete loss of antimicrobial properties of cephalosporins with the cleavage of the rings and determines the antimicrobial activity of cephalosporins.

Cephalosporins cefazolin (CEF), ceftriaxone (CTRX), moxalactam (MXLM), cefuroxime (CFXM), and cefotaxime (CFTM) are first, second, and third generation cephalosporin derivatives, which are widely used in clinical therapy of severe infections (2).

Cefazolin, which is a first generation of cephalosporin antibiotics, consist of β-lactam ring, is mainly used to treat bacterial infections of the lung, stomach, skin, bone, heart valve, and blood (3-4). Especially, it is clinically effective against infections caused by *Staphylococci* and *Streptococci* of gram-positive bacteria (5). Generally, the interaction of β-lactam antibiotics with metal ions is of a complex nature (6). Toxicological and pharmacological properties of many drugs have been changed when they are in the form of metal complexes.

In the present study, [Co(Cefaz)Cl] complex was synthesized via a procedure in the literature. Redox properties of the complex were determined with cyclic voltammetry and linear sweep voltammetry methods. The relationship between the theoretical calculation and antioxidant capacity was investigated to confirm the experimental findings.

EXPERIMENTAL SECTION

Synthesis

The synthesis of [Co(Cefaz)Cl] was achieved according to published literature (7-8). The [Co(Cefaz)Cl] complex was prepared by mixing 1 mmol $CoCl_2 .6H_2O$ salt and 2 mmol cefazolin ligand in methanol (MeOH). 0.5 M NaOH was added dropwise to adjust the pH of the solution to 8.0. The solution mixture was aproximately stirred for 2 h at room temperature. The precipitated complex was filtered off, washed with diethyl ether and dried in a desiccator at room temperature. The melting point of the complex was found to be higher than 360°C. The formed [Co(Cefaz)Cl] complex is highly soluble in dimethylsulfoxide (DMSO) and DMF.

Computational Details

The complete geometry optimizations of Cefaz and [Co(Cefaz)Cl] complex were performed using the Density Functional Theory (9). The Becke three parameter hybrid exchange functional and the Lee-Yang- Parr correlation functional (B3LYP) (10-11) with LanL2DZ basis set were used for all the atoms of Cefaz and [Co(Cefaz)Cl] complex in TD-DFT calculations of HOMO-LUMO energy and molecular orbital analyses. The results are reported in Table 4. All the energy calculations were performed by the GAUSSIAN 09 package program (12).

Quantum chemical descriptors were approximated in terms of the one-electron energies of the frontier molecular orbitals (FMO), HOMO and LUMO, E_H and E_L, using the following equations (1)–(5):

$$\Delta_{gap} = E_L - E_H \quad (1)$$

$$\mu = \frac{-(E_H + E_L)}{2} \quad (2)$$

$$\eta = \frac{E_L - E_H}{2} \quad (3)$$

$$\omega = \frac{\mu^2}{2\eta} \quad (4)$$

$$\chi = -\mu \quad (5)$$

Here, η is chemical hardness, μ is electronic chemical potential, ω is electrophilicity index, and χ is electronegativity (13-15).

Electrochemistry

Cyclic Voltammetry (CV) and Linear Sweep Voltammetry (LSV) experiments were carried out using an Ivium Vertex potentiostat/galvanostat system. A conventional three electrode cell consisting of glassy carbon (3.0 mm diameter) working electrode (GCE), Ag/AgCl reference electrode and a platinum wire counter electrode were used for all electrochemical experiments. The GCE used as working electrode was polished with alumina slurry (Buehler Micropolish) using a polishing pad (Buehler-102 mm) and rinsed with water before each run. Measurements were performed in $1x10^{-3}$ M [Co(Cefaz)Cl] complex solution in DMSO containing 0.1 M of tetrabutylammonium perchlorate (TBAP) as supporting electrolyte. Before all electrochemical measurements, the solutions were saturated with pure nitrogen gas for 10 min.

Antioxidant Efficiency Assays

CUPRAC Assay

1 mL of 10 mM $CuCl_2.2H_2O$, 1 mL of 7.5 mM Nc (neocuproine), 1 mL of 1.0 M pH 7 NH_4Ac buffer solution, x mL antioxidant sample solution and (1.1-x) mL H_2O were added to test tube, in this order. After the mixture, in a total volume of 4.1 mL, was incubated for 30 min, the absorbance spectra was recorded at 450 nm (16). The TEAC coefficients (trolox equivalent antioxidant capacities) were calculated from the ratio of the molar absorptivity of each compound to that of the trolox reaction (ε_{trolox}: 1.58×10^4 L mol^{-1} cm^{-1}).

DPPH Radical Scavenging Assay

The radical scavenging activities of the Cefaz ligand and [Co(Cefaz)Cl] complex were tested at different concentrations (12.5, 25, 37.5, 50, and 62.5 μM). Appropriate volume of the compound solution was mixed with 2 mL of 100 μM DPPH solution, and it made up to a final volume of 4 mL using methanol. After solution was stirred gently, the mixture was incubated for 30 min in the dark. The absorbance was measured at 515 nm (17) and the scavenging activity percentage was determined by the following equation:

DPPH radical scavenging activity (%) = $[(A_{control} – A_{sample})/ A_{control}] \times 100$

Where $A_{control}$ and A_{sample} are absorbance of control and sample, respectively. IC_{50} values were calculated from the graph plotted between DPPH radical scavenging activity percentages and different solution concentrations. Ascorbic acid was used as standard.

Table 1: ATR-FTIR spectral findings of the Cefaz and [Co(Cefaz)Cl] complex.

	v (N-H)	**v (C=O) lactam**	**v (C=O) amide**	**v (COO) asym**	**v (COO) sym**	**v (C-N)**	**v (C-O) stretch**
Cefaz	3289	1758	1667	1540	1386	1490	1008
[Co(Cefaz)Cl]	3287	1765	1672	1547	1390	1497	1013

RESULTS AND DISCUSSION

Infrared Spectrum

FT-IR data were obtained by an Agilent Cary 630 spectrometer with an Attenuated Total Reflectance (ATR) unit in the 4000–600 cm^{-1} range. A comparison of the ATR-FTIR spectral findings of the Cefaz and [Co(Cefaz)Cl] complex are shown in Table 1. The similar spectral values observed for Cefaz and [Co(Cefaz)Cl] complex in the ATR-IR spectra. The main vibrational frequency values are 3289 v(N-H), 1758 v(C=O)lactam, 1667 v(C=O)amide, 1540 v(COO)asym, 1386 v(COO)sym, 1490 v(C-N), 1008 v(C-O)stretch for Cefaz Ligand. FTIR spectral values of the [Co(Cefaz)Cl] complex are 3287 v(N-H), 1765 v(C=O)lactam, 1672 v(C=O)amide, 1547 v(COO)asym, 1390 v(COO)sym, 1497 v(C-N), 1013 v(C-O)stretch. Slight shifts (5-25 cm^{-1}) were observed in the spectrum of Cefaz and [Co(Cefaz)Cl] complex. Cobalt makes coordination bond with amide carbonyl group, COO group and nitrogen atoms. The coordination of metal atom is completed with the chloride ion. The ATR-FTIR spectral results of the Cefaz and [Co(Cefaz)Cl] complex proved the proposed structure. The results are in good agreement with literature (7-8).

Electrochemistry

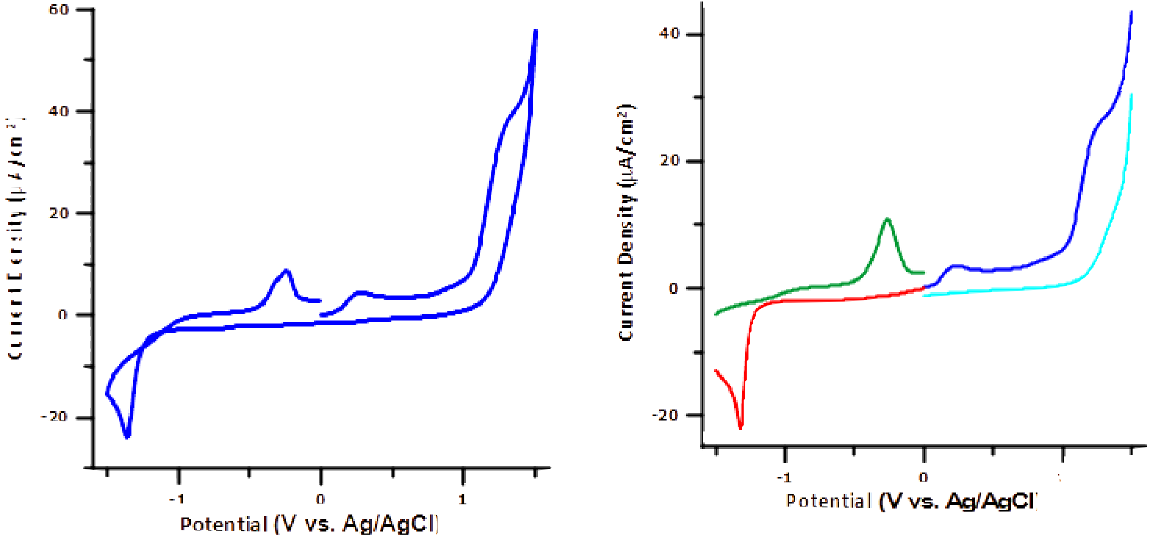


Figure 1. Left) CV and right) LSV of [Co(Cefaz)Cl] complex in 0.1 M TBAP/DMSO solution.

Electrochemical measurements of [Co(Cefaz)Cl] complex were used to estimate its electron transfer ability in DMSO. The CV and LSV of the complex were recorded in the potential range of (-1.5) – 1.5 V versus Ag/AgCl (Fig. 1). The [Co(Cefaz)Cl] complex showed three redox processes in DMSO. One quasi-reversible transfer process was observed with a reduction peak at E_{pc}=-1.36 V with a corresponding oxidation peak at E_{pa} = -0.24 V at a 50 mV/s scan rate in the CV curve of the complex (18). The peak separation (ΔEp) of this Co^{II}/Co^{I} couple is 1.1 V. Positive potential scans for [Co(Cefaz)Cl] showed two irreversible oxidative responses at 0.28 V and 1.27 V attributed to the metal based Co^{II}/Co^{III} and the ligand based (L/L^{+}) oxidative responses, respectively. Similar voltammetric responses were observed in the Linear Sweep voltammetry method (Fig 1b) for the complex.

Antioxidant Efficiency

The antioxidant efficiencies of Cefaz and [Co(Cefaz)Cl] complex were assayed by using the CUPRAC (cupric reducing antioxidant capacity) method. Antioxidant activity deals with reaction kinetics and the chemical oxidation rate of the antioxidant or the rate of quenching of the reactive species by the antioxidant (16,19). The TEAC coefficients of ascorbic acid used as a reference and compounds are shown in Table 2.

The order of in vitro antioxidant capacity of the compounds was [Co(Cefaz)Cl] > Cefaz in the assay (20). The antioxidant activity of Cefaz was found to be less than those of [Co(Cefaz)Cl] and Ascorbic Acid.

Table 2: The TEAC coefficients of the compounds.

Compounds	TEAC
Cefaz	0.80±0.058
[Co(Cefaz)Cl]	1.17±0.201
Ascorbic Acid	1.00 ± 0.01

The Cefaz and [Co(Cefaz)Cl] complex were tested for DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging activity. DPPH has a strong absorption bond at 515 nm due to having an odd electron. When this electron becomes paired off, the absorption value decreases stoichiometrically according to the number of electrons taken up (21).

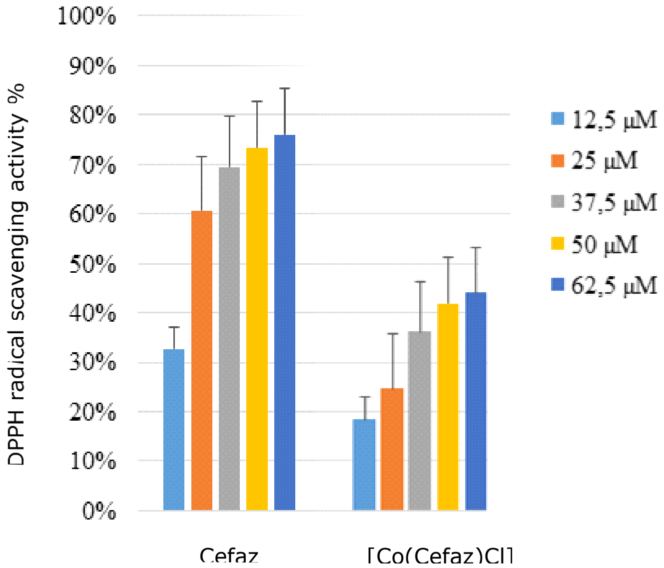


Figure 2. The free radical scavenging activity of the compounds (%).

Table 3. The radical scavenging activities of the compounds, as IC_{50}.

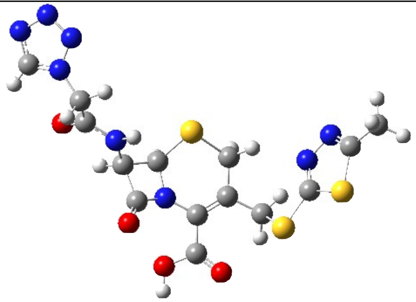
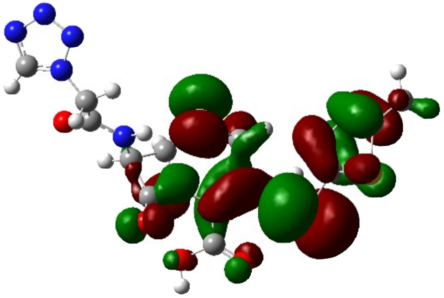
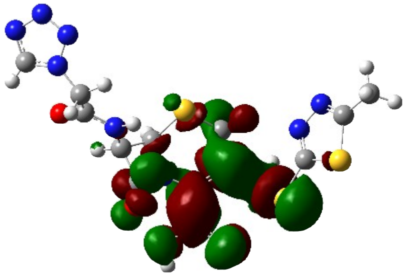
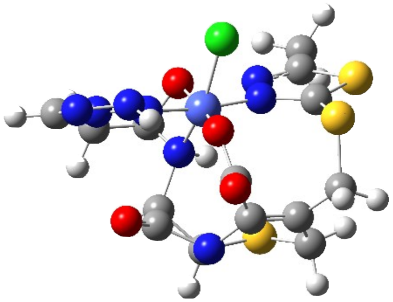
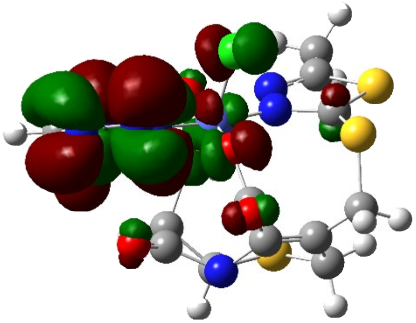
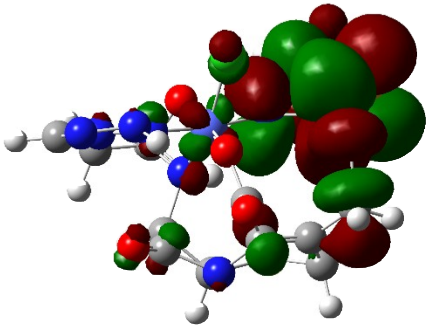
Compounds	DPPH IC_{50} (µM)
Cefaz	43.5
[Co(Cefaz)Cl]	136.8

A lower IC_{50} value indicates better scavenging activity.

According to this, Cefaz > [Co(Cefaz)Cl].
Cefaz ligand has moderate antioxidant activity when compared to ascorbic acid which used as standard. The DPPH radical scavenging activity results of Cefaz could not be supported by the TEAC value (0.80) obtained by the CUPRAC method. The differences between the two methods are due to the fact that the methods run with different reactions (22).

DFT Calculations

Table 4. Optimized geometry and frontier molecular orbital density distribution of the Cefaz and [Co(Cefaz)Cl] complex calculated at B3LYP/LanL2DZ.

	Structure	HOMO	LUMO
Cefaz			
[Co(Cefaz)Cl]			

The energy differences of the frontier orbitals of the donor and acceptor, which correspond to the charge transfer transition, ($\Delta E_{gap}=LUMO_{acceptor}-HOMO_{donor}$) (23) for the compounds were calculated from the HOMO and LUMO energies, which reflect the chemical activity of the molecule. The sample contour plots and 3D structures of the frontier orbitals (HOMOs and LUMOs) of the Cefaz and [Co(Cefaz)Cl] complex and their corresponding energy levels and gaps calculated with B3LYP/ LanL2DZ basis set are shown in Table 4.

The HOMO density for the Cefaz molecule is localized on the whole molecule surface, except for the some carbon and nitrogen atoms, and tetrazole group. The LUMO is distributed on the locally oxygen and sulfur heteroatoms and aromatic ring. On the other hand, as can be seen from Table 4, the LUMO extends over thiadiazole group and locally chlorine atom on the molecular surface for the [Co(Cefaz)Cl] complex. However, HOMO is located mainly on the chlorine atom and tetrazole group on the molecule surface. According to this findings, heteroatoms can be regarded as the active sites of compounds.

Table 5. Some Quantum chemical descriptors of the Cefaz and [Co(Cefaz)Cl] compounds calculated at B3LYP/LanL2DZ.

	E_{HOMO} (eV)	**E_{LUMO} (eV)**	**ΔE_{gap}**	**ω(eV)**	**μ (eV)**	**η (eV)**	**χ**
Cefaz	-6.974	-2.768	4.206	5.641	4.871	2.103	-4.871
[Co(Cefaz)Cl]	-4.460	-2.780	1.680	7.800	3.620	0.840	-3.620

The effect of the complexation reaction was investigated theoretically in determining both the antioxidant and electronic properties of the compounds. The HOMO-LUMO energy gap (ΔE_{gap}) of [Co(Cefaz)Cl] complex was found to be lower than Cefaz ligand (Table 5). It is estimated that the nitrogen atoms increase the HOMO density on the substituted tetrazole ring and also cause a decrease in ΔE_{gap} by raising the HOMO energies of the complex (Table 4). Accordingly, the [Co(Cefaz)Cl] complex can be expected to show greater reactivity, and higher antioxidant properties were observed in the experimental studies for the complex (Table 2).

On the other hand, the complexation reaction increases the electrophilic index (ω) and electronegativity (χ) of the synthesized [Co(Cefaz)Cl] complex. Therefore [Co(Cefaz)Cl] complex was found to be more reactive than Cefaz ligand in terms of antioxidant properties.

CONCLUSION

Electrochemical measurements of the [Co(Cefaz)Cl] complex show that the complex has two irreversible metal- and ligand-centered one-electron transfer response on the oxidation side and one quasi-reversible $Co^{II/I}$ couple indicating metal centered one-electron transfer on the reduction side in the CV and LSV voltammograms. The antioxidant activity of Cefaz was found to be less than [Co(Cefaz)Cl] complex and ascorbic acid. The HOMO-LUMO energy gap (ΔE_{gap}) of [Co(Cefaz)Cl] complex was found to be lower than Cefaz ligand. According to the theoretical calculations, [Co(Cefaz)Cl] complex is expected to exhibit greater reactivity. The higher antioxidant property was observed for this complex in the experimental studies. The experimental results are in good agreement with the theoretical calculations.

CONFLICT OF INTEREST

No conflict and interest.

ACKNOWLEDGMENTS

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