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Analysing The Effect Of Metal Complexes With Cefuroxime On Some Selected Bacteria

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Abstract

Silver and its compounds have long been utilized as antimicrobial agents in medicine. Silver sulfadiazine, a broad-spectrum antibiotic ointment, is widely used and effective against a wide range of bacteria and some yeast. Copper and its alloys possess natural antimicrobial properties. Ancient civilizations recognized and utilized the antimicrobial effects of copper long before the understanding of microbes in the nineteenth century. Mechanochemistry refers to reactions induced by the input of mechanical energy, typically involving solids, such as grinding in ball mills. It is gaining increased attention due to its ability to promote rapid and quantitative reactions between solids, often without the need for additional solvents. Historically, mechanochemistry has been a secondary approach to chemical synthesis, with solution-based methods being the default choice. Building upon our previous research on antibiotic resistance, this paper presents the impact of mechanochemically synthesized copper (II) and silver (I) complexes with cefuroxime on cephalosporin-resistant bacteria.

Key Words: Mechanochemistry, Cu and Ag Complex, Cefuroxime, Anti-microbial activity.

Introduction

The treatment of diseases remains a significant and complex issue due to various factors, including the emergence of infectious diseases and the rise in multi-drug resistant microbial pathogens. Despite the availability of numerous antibiotics and chemotherapeutics for medical use, the past few decades have witnessed the emergence of both old and new antibiotic resistance, highlighting the need for the discovery of new compounds with antimicrobial activity. These compounds may act through mechanisms that differ from those of well-known classes of antimicrobial agents, to which many clinically relevant pathogens have developed resistance. Antibiotic resistance occurs when bacteria undergo changes that reduce or eliminate the effectiveness of drugs or chemicals designed to treat or prevent infections. As a result, bacteria can survive and continue to multiply, causing further harm. Bacteria employ various mechanisms to achieve this, including neutralizing the antibiotic, pumping it out rapidly, or altering the site of attack to render the antibiotic ineffective. Therefore, the development of antimicrobial agents capable of addressing this resistance problem remains a top priority for synthetic chemists. Treating resistant microbes is increasingly challenging, often requiring alternative medications or higher doses. Consequently, there is a growing need for new antibiotic therapies, although the development of new drugs is becoming less common. Cephalosporins, which are bactericidal and share a similar mode of action with other β-lactam antibiotics like penicillin, are less susceptible to β-lactamases. They disrupt the synthesis of the peptidoglycan layer, which forms the bacterial cell wall and is crucial for maintaining structural integrity.The synthesis of peptidoglycan concludes with the final transpeptidation step, which is facilitated by penicillin-binding proteins (PBPs) known as transpeptidases. Resistance to cephalosporin antibiotics can occur through either a decrease in affinity of existing PBP components or the acquisition of an additional β-lactam-insensitive PBP. Currently, certain strains of Citrobacter freundii, Enterobacter cloacae, Neisseria gonorrhea, and Escherichia coli have developed resistance to cephalosporin. Similarly, varying degrees of resistance to cephalosporin have been observed in strains of Morganella morganii, Proteus vulgaris, Providencia rettgeri, Pseudomonas aeruginosa, and Serratia marcescens. Silver and its compounds have long been utilized as antimicrobial agents in medicine. Silver sulfadiazine, a broad-spectrum antibiotic ointment, is widely used and effective against a wide range of bacteria and some yeast. Copper and its alloys possess natural antimicrobial properties. Ancient civilizations recognized and utilized the antimicrobial effects of copper long before the understanding of microbes in the nineteenth century. Mechanochemistry refers to reactions induced by the input of mechanical energy, typically involving solids, such as grinding in ball mills. It is gaining increased attention due to its ability to promote rapid and quantitative reactions between solids, often without the need for additional solvents. Historically, mechanochemistry has been a secondary approach to chemical synthesis, with solution-based methods being the default choice. Building upon our previous research on antibiotic resistance, this paper presents the impact of mechanochemically synthesized copper (II) and silver (I) complexes with cefuroxime on cephalosporin-resistant bacteria.

Materials and Method

All the chemicals utilized were of analytical grade and were acquired from Bristol Scientific Company Limited. They were employed without any additional purification. The ligand employed in the experiment was cefuroxime (Cfu), while the metals used were copper chloride dihydrate [CuCl2.2H2O] and silver nitrate [AgNO3]. In order to obtain the IR spectra of the complexes, KBr pellets were utilized and the range of 4000-400 cm-1 was examined using an FTIR spectrometer. The metal analysis was determined through atomic absorption spectroscopy using a Perkin-Elmer Spectrometer, model 3110. UV-Vis spectra were obtained using a UV-2550 Shimazu Spectrophotometer within the wavelength range of 200-800 nm. The synthesis of the complexes was carried out by modifying the literature procedure and employing the mechanochemical method. For the synthesis, cefuroxime (10 mmol, 4.25 g) and copper chloride dihydrate (10 mmol, 1.705 g) were accurately weighed and transferred into a mortar. The two reactants were then ground for a duration of twenty (20) minutes to obtain a homogeneous powder. The powder was subsequently removed from the mortar and stored in a desiccator. The same procedure was followed for silver nitrate (10 mmol, 1.699 g) and cefuroxime (10 mmol, 4.25 g).

Equation for reaction

$CuCl_2.2H_2O + CFU \rightarrow [Cu(CFU)2H_2O] + Cl_2$ $AgNO_3 + CFU \rightarrow [Ag(CFU)NO_3]$
Where CFU = Cefuroxime

Anti microbial screening

The disc diffusion method was employed to assess the in-vitro antimicrobial activities of antibiotics and their metal complexes against various microorganisms. Strepto coccus pneumoniae, Bacillus subtillu, Salmonella typhi, Klebsielia pnuemoniae, Escherichia coli, Methicillin- resistance staphylococcus aureus (MRSA), Pseudomonas aeruginosa, and Staphlylococcus aureus were the microorganisms tested. Each microorganism was added to a sterile nutrient agar medium, which was then spread on sterile Petri dish plates and allowed to solidify. Different concentrations (30, 20, and 10 mg/mL) of antibiotics and their metal complexes in methanol were placed on the culture media and incubated at 37◦C for 24 hours. The diameter of the zone of inhibition (mm) was measured to determine the activities. Antibiotics and their complexes that exhibited a zone of inhibition of 10 mm or more underwent further testing for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using samples with concentrations of (6, 4, and 2) mg/mL in methanol, using the same bacterial species in peptone water.

Result and discussion

The copper and silver ion complexes obtained exhibit different physical properties. The copper complex appears as an air stable light green powder, while the silver complex is a white powder. Both complexes show solubility in polar solvents like distilled water, methanol, ethanol, and dimethylsulfoxide (DMSO). This solubility indicates that the compounds are likely polar in nature. A similar observation was made by another researcher. The melting point of the copper complex is 110°C, while the silver complex has a melting point of 1200°C (Table 1). The difference in melting points between the ligand and the complexes suggests the formation of new compounds and provides evidence of complexation. The molar conductivity of the complexes ranges from 3.6 to 4.5 Scm2/mol (Table 1), indicating that the complexes are non-electrolytes.

Infrared Spectra

The infrared spectra data for both the complexes and their ligand can be found in Table 2. The band assignments were determined by comparing them with previous studies on mixed ligand complexes and drug-based metal complexes. In the free ligand, the vibrations around 3190 cm-1 were identified as v(O-H) stretching frequency, but upon complexation, these vibrations shifted in the complexes. The band at 3560 cm-1 in the free ligand was also attributed to v(N-H2) vibration of the amine group. Additionally, the band at 1550 cm-1 was assigned to v(C=N) vibration, which was also observed by other researchers.

In the spectra of the free ligand, a strong intensity band associated with v(C=O) vibration stretching was observed at 1720 cm-1. Similar bands were observed in the metal complexes, but with a lower wavelength shift compared to the ligand. However, their intensities were reduced (as shown in Table 2). The appearance of new bands at 620 and 630 cm-1 in the spectra of the complexes, which can be attributed to v(M-O) stretching, suggests the formation of the complexes.

Electronic spectra

The spectral data for cefuroxime and its complexes can be found in Table 3. Previous studies have assigned the transition at 349 nm in the cefuroxime spectra to a $\pi \rightarrow \pi^*$ transition (Table 3). This finding is consistent with similar observations reported in the literature. The [Cu(CFU)2H2O] complex exhibited a low intensity band at 340 nm, which was attributed to MLCT. In the case of the [Ag(CFU)NO3] complex, absorption bands were observed at (287, 301, and 313) nm, indicating a bathochromic shift compared to the free ligand. This suggests a weak interaction between the ligand and silver ion, which can be attributed to MLCT.

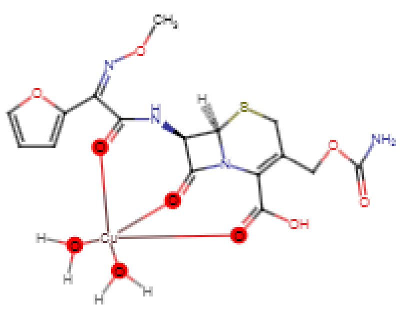
Micro analysis

Table 4 presents the microanalysis of the metal complexes. The findings indicate a satisfactory correlation between the % C, H, and N values and the suggested structures. Based on the acquired data, it is evident that the analyzed complexes correspond to [Cu (L) 2H2O] and [Ag (L) NO3], where L= CFU.

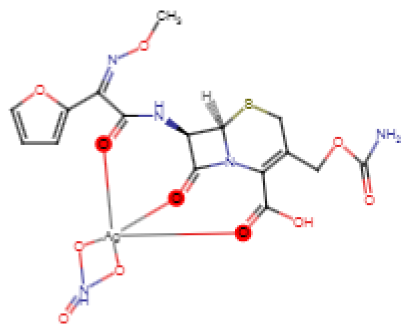
Anti microbial studies

Transition metal complexes are of great importance in the field of biological study. Several of these complexes have been extensively researched due to their antimicrobial and anticancer properties. The investigation of metal complexes has yielded significant findings (Table 5). Notably, a novel study has focused on the antimicrobial activities of Cu (I) and Ag (I) complexes. Building upon this discovery, the current study aimed to synthesize new Cu (II) and Ag (I) complexes with cefuroxime using a mechanochemical method. The antimicrobial effects of these complexes were also evaluated.

Structure of the complex



Cu Complex



Ag Complex

Table 1: Analytical data of cefuroxime and its complexes

Compounds	Molecular formula (Molar mass)	Color	Yield (g) (%)	M.pt (^{0}C)	Conductivity (Scm^2/mol)	TLC (RF Values)
CFU	$6H_{16}N_4O_8S$(424.39)	White	-	218	-	0.4
[Cu(CFU)2H_2O]	[Cu($C_{16}H_{20}N_4O_{10}S$] (523.89)	Light green	5.61 (94.0)	120	4.5	0.8
[Ag(CFU)NO_3]	[Cu($C_{16}H_{16}N_5O_{11}S$] (594.76)	White	5.82 (98.0)	110	3.6	0.6

Table 2: Infrared spectral data of cefuroxime and its metal complexes

Compounds	ν(O-H) (cm^{-1})	ν(N-H) (cm^{-1})	ν(C=O) (cm^{-1})	ν(NH_2) (cm^{-1})	ν(C=N) (cm^{-1})	ν(C-S) (cm^{-1})	ν(C=C) (cm^{-1})	ν(M-O) (cm^{-1})
CFU	3190	1872	1720	3560	1550	2050	1235	-
[Cu(CFU)2H_2O]	3235	1890	1700	3451	1500	2030	1245	620
[Ag(CFU)NO_3]	3120	1865	1680	3473	1570	2040	1250	630

Table 3: UV-Vis spectra of cefuroxime and its metal complexes

Ligand/Complexes	Formula	Wavelength (nm)	Energies (cm^1)	Assignment
CFU	$C_{16}H_{16}N_4O_8S$	349	2865	$\pi\rightarrow\pi^*$
[Cu(CFU)2H_2O]	[Cu($C_{16}H_{20}N_4O_{10}S$]	340	2941	MLCT
[Ag(CFU)NO_3]	[Cu($C_{16}H_{16}N_5O_{11}S$]	287 301 313	3484 3322 3195	$n\rightarrow\pi^*$ MLCT MLCT

Table 4: Microanalysis of Cu(II) and Ag (I) complexes

Compounds	Molecular formula (Molar mass)	Microanalysis: found (calculated)%			
		C	H	N	M
[Cu(CFU)2H_2O]	[$CuC_{16}H_{20}N_4O_{10}S$] (523.89)	36.62 (36.65)	3.80 (3.82)	10.62 (10.69)	12.15 (12.12)
[Ag(CFU)NO_3]	[$AgC_{16}H_{16}N_5O_{11}S$] (594.76)	32.01 (32.28)	2.50 (2.69)	11.75 (11.77)	18.17 (18.14)

Table 5: Antimicrobial activities of cefuroxime and its metal complexes

Compounds	**Conc. mg/mL**	***MRSA***	***S.aureus***	***S.pneumoniae***	***B.subtilis***	***E.coli***	***S.typhi***	**K.*pneumoniae***	***p.aeruginosa***
CFU	10	7.0±0.8	10±0.5	0.0±0.0	12±0.5	0.0±0.0	10±0.4	0.0±0.0	0.0±0.0
	20	11±0.2	11±0.6	0.0±0.0	14±0.3	0.0±0.0	13±0.6	0.0±0.0	0.0±0.0
	30	14±0.5	13±0.4	0.0±0.0	18±0.6	0.0±0.0	16±1.0	0.0±0.0	0.0±0.0
(CFU)2H_2O]	10	9.0±0.8	11±0.3	0.0±0.0	13±0.4	0.0±0.0	11±0.5	0.0±0.0	0.0±0.0
	20	11±0.7	14±0.8	0.0±0.0	16±0.3	0.0±0.8	16±0.4	8.0±0.0	0.0±0.0
	30	15±0.4	17±0.8	0.0±0.0	23±1.0	0.0±0.9	22±0.3	11±0.0	0.0±0.0
[Ag (CFU)NO_3]	10	9.0±0.1	11±0.2	0.0±0.0	13±0.0	0.0±0.0	8.0±0.3	0.0±0.0	0.0±0.0
	20	11±0.9	14±0.1	0.0±0.0	17±0.5	0.0±0.0	12±0.3	8.0±0.7	7.0±0.4
	30	15±0.2	17±1.0	0.0±0.0	23±0.4	0.0±0.0	15±0.5	11±0.6	9.0±0.4

MRSA= Methicillin-resistance *staphylococcus aureus, s.aureus = staphylococcus aureus, s.pneumoniae = Strepto coccus pneumonia, B.subtilis=Bacillus subtilis, E.coli= Escherichia coli, S.typhi= Salmonella typhi, K.pneumoniae=Klebsiellapneumonia and P.aruginosa= Psuedomonas aeruginosa.*

Table 6: Minimum inhibitory concentration (MIC) of cefuroxime and its metal complexes

Compounds	**Conc. mg/mL**	***MRSA***	***S.aureus***	***B.subtilis***	***S.typhi***	**K.*pneumoniae***	***aeruginosa***	***E.coli***	***S.pneumoniae***
CFU	1	R	R	R	R	NA	NA	NA	NA
	2	R	R	R	R	NA	NA	NA	NA
	4	R	R	R	R	NA	NA	NA	NA
	6	R	S	S	S	NA	NA	NA	NA
	8	S	S	S	S	NA	NA	NA	NA
	10	S	S	S	S	NA	NA	NA	NA
[Cu(CFU)2H_2O]	1	R	R	R	R	NA	NA	NA	NA
	2	R	R	R	R	NA	NA	NA	NA
	4	R	R	R	R	NA	NA	NA	NA
	6	R	S	S	R	NA	NA	NA	NA
	8	S	S	S	S	NA	NA	NA	NA
	10	S	S	S	S	NA	NA	NA	NA
[Ag(CFU)NO_3]	1	R	R	R	R	NA	R	R	R
	2	R	S	R	R	NA	R	R	R
	4	R	S	S	R	NA	R	R	S
	6	R	S	S	S	NA	S	S	S
	8	R	S	S	S	NA	S	S	S
	10	S	S	S	S	NA	S	S	S

R= resistant, S= susceptible and NA= not applicable

From the result of minimum inhibitory concentration (MIC), it appears that both the ligand and the complexes have MIC of 6 and 8 mg/mL on *MRSA*, *s. aureus*, *B. subtilis* and *S. typhi*. However, [Ag(CFU)NO_3] has MIC of 4mg/mL on *S. pneumoniae* and 6 mg/mL on both *E.coli and P.aeruginosa* (Table 6).

Table 7: Minimum Bactericidal concentration (MBC) of cefuroxime and its metal complexes

Compounds	**Conc. mg/mL**	***RSA***	***ureus***	***btilis***	***S.typhi***	**K.*pneumoniae***	***aeruginosa***	***E.coli***	***S.pneumoniae***
CFU	2	R	R	R	R	NA	NA	NA	NA
	4	R	R	R	R	NA	NA	NA	NA
	6	R	S	S	S	NA	NA	NA	NA
	8	S	S	S	S	NA	NA	NA	NA
	10	S	S	S	S	NA	NA	NA	NA
[Cu(CFU) 2H_2O]	2	R	R	NA	R	NA	NA	NA	NA
	4	R	R	NA	R	NA	NA	NA	NA
	6	R	S	NA	R	NA	NA	NA	NA
	8	S	S	NA	R	NA	NA	NA	NA
	10	S	S	NA	S	NA	NA	NA	NA
[Ag(CFU)NO_3]	2	R	R	R	R	R	R	R	R
	4	R	R	R	R	R	R	R	R
	6	R	S	R	R	R	R	R	S
	8	R	S	S	S	R	R	S	S
	10	S	S	S	S	S	S	S	S

The MBC result also shows that both the ligand and the complexes have MBC ranging from 6-10 mg/mL on microorganism tested (Table 7).

Conclusion

Five coordinated complexes were suggested based on the findings derived from the analysis of both compounds. The assessment of inhibition zones for the ligand and complexes revealed that the prepared complexes exhibit improved antibacterial efficacy against cephalosporin-resistant bacteria compared to the ligand.

Contribution Declaration

1. Worked on the introduction part and the complex formation work.
2. Collected data for spectroscopic analysis
3. Write up work for the antimicrobial study and micro analysis of complex
4. Write up work for antimicrobial study and monitored antimicrobial study
5. Collection of literature review and other related documents
6. Monitored overall document work and set up data

Ethical Approval

i)Not applicable

Funding

i) Not applicable

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