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Research Article

Mechanochemically Synthesized Copper (II) and Silver (I) Complexes with Cefuroxime: A Promising Strategy to Combat Cephalosporin-Resistant Bacteria

Maryam javaid, Kainat Javeed

Department of Chemistry, The faculty of chemical and biological Sciences, The Islamia University Of Bahawalpur, Pakistan

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ABSTRACT

Pharmaceutical drug development frequently involves the use of complexation to modify the pharmacological, toxicological, and physico-chemical properties of drugs. In our investigation, we employed solvent-free (mechanochemical) synthesis techniques to create copper (II) and silver (I) complexes with cefuroxime. Various physico-chemical analyses, including infrared spectroscopy, UV/Visible spectroscopy, elemental analysis, melting point determination, solubility tests, and conductivity measurements, were utilized to characterize these complexes. Our findings led to the proposed chemical formulas of [Cu(CFU)2H2O] and [Ag(CFU)NO3], where CFU represents cefuroxime. To assess the antimicrobial activity of the synthesized complexes, we conducted experiments using the disc diffusion method against a range of bacteria, including Streptococcus pneumonia, Bacillus subtillus, Salmonella typhi, Klebsiella pneumoniae, Escherichia coli, Methicillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa, and Staphylococcus aureus. Our results demonstrated that these complexes exhibited superior antimicrobial activity compared to the free ligand. Analysis of the IR spectra indicated that cefuroxime coordinated with the metal ions through v(COO), v(C=O), and the oxygen atom of the water molecule. Moreover, distinct differences in the melting point, color, and electronic spectra of the complexes in comparison to the ligand strongly suggest the formation of coordination compounds.

INTRODUCTION

Due to a number of causes, such as newly emerging infectious diseases and the rise of multi-drug-resistant microbial infections, treating diseases remains a significant and difficult problem. Despite the abundance of antibiotics and

***Corresponding Author:** Maryam javaid
Address: *Department of Chemistry, The faculty of chemical and biological Sciences, The Islamia University Of Bahawalpur, Pakistan*
Email : maryamjavaidfts@gmail.com

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chemotherapeutics that are available for medical use, the emergence of both old and new antibiotic resistance observed in the last few decades revealed a significant amount of new compounds endowed with antimicrobial activity, possibly acting through a mechanism of action that is different from those of well-known classes of antibiotic agents to which many clinically relevant pathogens are now resistant [1]. When bacteria adapt in a way that lessens or eliminates the effectiveness of medications, chemicals, or other agents intended to treat or prevent illnesses, this phenomenon is known as antibiotic resistance. The bacteria persist, reproduce, and cause further damage. Bacteria have a number of ways to accomplish this. Some bacteria can become immune to the antibiotic before it can cause any harm; others can rapidly pump the antibiotic out; and still others can change the antibiotic attack site so it cannot affect the function of the bacteria. Therefore, the provision of antimicrobial agents that can tackle this resistance problem remains the priority of synthetic chemists [2]. Resistance microbes are increasingly difficult to treat, requiring alternative medication or a higher dose. Hence, calls for new antibiotic therapies have been made, but new drug development is becoming rarer [3]. Cephalosporins are bactericidal and have the same mechanism of action as other β-lactam antibiotics (eg, penicillin), but are less sensitive to β-lactamases than β-lactam antibiotics. Cephalosporin prevents the peptidoglycan layer that makes up the bacterial cell wall from synthesizing properly. For the structural integrity of the cell wall, the peptidoglycan layer is critical. Transpeptidases known as penicillin-binding proteins (PBPs) promote the last transpeptidation step in the formation of the peptidoglycan [4]. It is possible to develop a new, -lactam-insensitive PBP or lessen the affinity of the current PBP components in order to resist cephalosporin antibiotics. Currently, some strains of Escherichia coli, Citrobacter freundii, Enterobacter cloacae, and Neisseria gonorrhoea are cephalosporin resistant. Various strains of Pseudomonas aeruginosa, Serratia marcescens, Proteus vulgaris, Morganellamorganii, and Providencia rettgeri have also developed resistance to cephalosporin [5]. In medicine, silver and its derivatives have long been employed as antibacterial agents. Silver sulfadiazine is a widely used broad-spectrum antibiotic ointment, effective against a broad range of bacteria and some yeast [1]. Copper and its alloys are natural antimicrobial material. Ancient civilizations exploited the antimicrobial properties of copper long before the concept of microbe became understood in the nineteenth century [6].

Mechanochemistry refers to reactions, normally of solids, induced by the input of mechanical energy, such as by grinding in ball mills. Its ability to facilitate solid-solid reactions fast and quantitatively with either no additional solvent or very little solvent has led to increased research interest. Historically, it has been a sideline approach to chemical synthesis, and solution-based methods have been adopted by default [7]. In continuation of our work on antibiotic resistance [8], this paper reports the effect of mechanochemically synthesized copper (II) and silver (I) complexes with cefuroxime on some cephalosporin resistant bacteria.

MATERIALS AND METHODS

Analytical-grade chemicals were utilized throughout. These were purchased from Bristol Scientific Company Limited and utilized directly. Cefuroxime (Cfu) is the ligand, and copper chloride dihydrate [CuCl2.2H2O] and silver nitrate [AgNO3] are the metals. Using an FTIR spectrometer, IR spectra of the complexes in KBr pellets were obtained in the 4000-400 cm-1 region. Using a Perkin-Elmer Spectrometer, model 3110, atomic absorption spectroscopy was used to analyze the metal. On the UV-2550 Shimazu

Spectrophotometer, UV-Vis spectra in the 200-800 nm wavelength range were acquired.

Synthesis of the Complexes

The literature process [9] was adapted and applied to the mechanochemical production of all the metal complexes. Carefully weighed substances were added to a mortar, including cefuroxime (10 mmol, 4.25 g) and copper chloride dihydrate (10 mmol, 1.705 g). To create a homogeneous powder, the two reactants were then crushed (grinding) for twenty (20) minutes. The powder was taken out of the mortar and put into a desiccator for storage. The same process was used to analyze cefuroxime (10 mmol, 4.25 g) and silver nitrate (10 mmol, 1.699 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

Antimicrobial Screening

Using the disc diffusion method, the antibiotics' in-vitro antimicrobial activities were evaluated against the following microorganisms: Streptococcus pneumoniae, Bacillus subtillus, Salmonella typhi, Klebsiella pneumoniae, Escherichia coli, Methicillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa, and Staphylococcus aureus. Each microorganism's suspension was mixed with a sterile nutrient agar medium before being distributed on sterile Petri dish plates and given time to set. Different antibiotic and metal complex doses (30, 20 and 10) mg/mL in methanol were applied to the culture media and incubated for 24 hours at 37 C. By taking a measurement of the zone of inhibition's diameter (mm), activities were calculated. Further testing was done on the antibiotics and their complexes that displayed zones of inhibition of 10 mm or more were further assayed for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using samples concentration of (6,4 and 2) mg/mL in methanol using same bacterial species in peptone water [10].

RESULTS AND DISCUSSIONS

The produced complexes of the copper and silver ions are white and light green powders, respectively, that are air stable. In polar solvents such distilled water, methanol, ethanol, and dimethylsulfoxide (DMSO), both complexes are soluble. Because the complexes are soluble in polar solvents, it is likely that the compounds are polar. Similar findings were noted by [11]. According to Table 1, the melting points of the copper and silver complexes are 110 and 1200C, respectively. The difference between the melting points of the ligand and the complexes suggests complexation as well as the possible creation of novel compounds [12]. The complexes' molar conductance ranges from 3.6 to 4.5 Scm2/mol (Table 1). The complexes may not be electrolytes, as indicated by this [13].

Infrared spectra

Table 2 shows the infrared spectrum data of the complexes and their ligands. Group assignments are determined for comparison with studies of mixed ligand complexes and some drug-based metal complexes [11]. The V (O-H) stretching frequency associated with vibrations centered at about 3190 cm-1 in the free ligand changes in the complexes after complex formation. The v(N-H2) vibration of the amine group was also due to the 3560 cm-1 band in the free ligand. The V (C=N) vibration was assigned to the band at 1550 cm-1. A similar observation was also noted by some workers [14]. In the spectrum of the free ligand, there was a strong intensity band at 1720 cm-1 due to vibrational stretching of v (C=O). In the metal complexes, the corresponding bands were observed with a smaller wavelength shift compared to the decrease in the intensity of the ligand pair (Table 2). The appearance of new bands at 620 and 630 cm-1 in the spectra of

complexes attributed to v(M-O) stretching indicates the formation of complexes.

Electronic spectra

The electronic spectral data of cefuroxime and its complexes are presented in Table 3. Based on previous determinations of related complexes [15-17]. The transition around 349 nm in the spectrum of cefuroxime (CFU) was assigned as a $\pi \rightarrow \pi^*$ transition (Table 3). A similar observation was made in previous literature [17]. The complex [Cu(CFU)2H2O] showed a low-intensity band at 340 nm, which was assigned to MLCT. The [Ag(CFU)NO3] complex showed an absorption band at wavelengths (287, 301 and 313) nm, indicating a bathochromic shift with respect to the free ligand and a weak interaction between the ligand and the silver ion, which can be attributed. To MLCT [16].

Microanalysis

The microanalysis of the metal complexes is presented in Table 4. The results revealed that the % C, H and N are in good agreement with the proposed structures. From the data obtained, it appears that the complexes analyzed as [Cu (L) $2H_2O$] and [Ag (L) NO3]. Where L= CFU.

Antimicrobial studies

Transition metal complexes play an important role in biological research. Some of them have now been widely studied for their antimicrobial and anticancer properties [15], and there is extensive research in the field of metal complexes [18]. New complexes of Cu(I) and Ag(I) have also been studied for their antimicrobial activity [16]. As a continuation of this discovery, new complexes of Cu (II) and Ag (I) with cefuroxime were synthesized in this study by a mechanochemical method, and the antimicrobial activity was monitored to determine whether the compounds in this study have activity or not . In this study, both the ligand and complexes were evaluated against both Gram-positive and Gram-negative bacteria such as: Strepto coccus pneumoniae, Bacillus subtillus, Salmonella typhi, Klebsielia pnuemoniae, Escherichia coli, MRSA, Pseudomonas aeruginosa and Staphlylococcus .Table 5 shows the results of inhibition zones of selected bacteria due to the action of the mediator and its complexes. The obtained results showed that the complexes were more effective against the microorganism than the ligand. The data also showed that the prepared complexes, followed by Staphlyococcus aureus, inhibited Bacillus subtillus to the highest degree. Although the ligand and complexes did not inhibit Escherichia coli and Pseudomonas aeruginosa at any concentration (Table 5). The complexes also inhibited Klebsiella pneumoniae at 20 and 30 mg/ml compared to the ligand, which showed less activity at the same concentration.

Structure of the complexes

The analytical data of this study revealed that coordination of cefuroxime to the metal ions occurs through oxygen atom of the carboxylate anion, oxygen atom of water molecule and oxygen atom of carbonyl for both complexes to give a coordination number of five. (Fig 1 and 2). This is similar to our previous report [8].

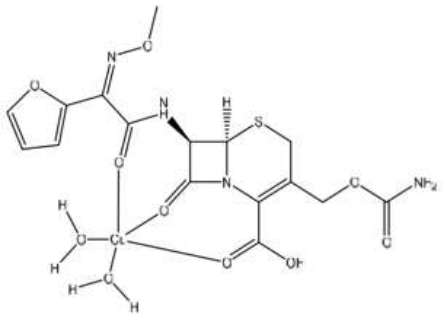

Figure 1: Copper complex of cefuroxime

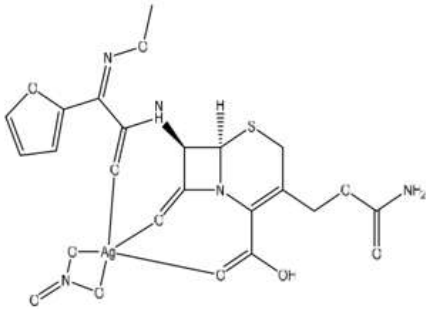

Figure 2: Silver complex of cefuroxime

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	C16H16N4O8S (424.39)	White	-	218	-	0.4
[Cu(CFU)2H_2O]	[Cu(C16H20N4O10S] (523.89)	Light green	5.61 (94.0)	120	4.5	0.8
[Ag(CFU)NO_3]	[Cu(C16H16N5O11S] (594.76)	White	5.82 (98.0)	110	3.6	0.6

CFU= Cefuroxime

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	C16H16N4O8S	349	2865	π→π*
[Cu(CFU)2H_2O]	[Cu(C16H20N4O10S]	340	2941	MLCT
[Ag(CFU)NO_3]	[Cu(C16H16N5O11S]	287 301 313	3484 3322 3195	n→π* 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]	[CuC16H20N4O10S] (523.89)	36.62 (36.65)	3.80 (3.82)	10.62 (10.69)	12.15 (12.12)
[Ag(CFU)NO_3]	[AgC16H16N5O11S] (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
[Cu(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*

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

Compounds	Conc mg/ml	MRSA	S.aureu	B.subtili	S.typhi	K. pneumonia	p. aeruginosa	E. coli	S. Pneumonia
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)2H2O	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	R	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)NO3]	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	*MRSA*	*S.aureus*	*B.subtilis*	*S.typhi*	K.*pneumoniae*	*p.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

Based on the results obtained from the analysis of both compounds, five coordinated complexes were proposed. Measurements of inhibition zones of the ligand and complexes showed that the prepared complexes have enhanced antibacterial activity on the cephalosporin resistance bacteria than the ligand.

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