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Effect of Mechano chemically Synthesized Copper (II) and Silver (I) Complexes with Cefuroxime on Some Cephalosporin Resistance Bacteria

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Abstract- Complexation plays a vital role in drug development as a means of modifying the pharmacological, toxicological and physico-chemical properties of drugs. In this study, Copper (II) and Silver (I) complexes of cefuroxime were synthesized by solvent free technique (mechano chemical). The complexes were characterized by physico-chemical methods such as infrared, UV/Visible, elemental analysis, melting point, solubility and conductivity. Based on the results obtained, the complexes were proposed to have the formula [(CFU)2H2O] and [Ag(CFU)NO3] where CFU stand for cefuroxime. The antimicrobial activities of the synthesized complexes were tested using disc diffusion method, against different strains of bacteria such as Strepto coccus pneumonia, Bacillus subtillus, Salmonella typhi, Klebsiella pnuemoniae, Escherichia coli, Methicillinresistance staphylococcus aureus (MRSA) ,Pseudomonas aeruginosa and Staphlylococcus aureus. It has been observed that the complexes have higher activity than the free ligand. The IR spectral data indicates that CFU coordinate to the metal ion through v(COO), v(C=O) and oxygen atom of water molecule. The melting point, colour and electronic spectra of the complexes were different from that of the ligand, which suggest formation of coordination compounds. Keywords: Antibiotic resistance, Cephalosporin, Silver, Copper and Mechanochemical.

1. INTRODUCTION

The treatment of diseases remains an important and challenging problem because of a combination of factors including emerging infectious diseases and the increasing number of multi-drug resistance microbial pathogens. Despite the large number of antibiotics and

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chemotherapeutics available for medicinal use, at the same time the emergence of old and new antibiotic resistance witnessed in the last decades revealed a substantial discovery of new compounds endowed with antimicrobial activity, possibly acting through mechanism of action, which is distinct from those of well-known classes of antimicrobial agents to which many clinically relevant pathogens are now resistant [1]. Antibiotic resistance occurs when bacteria change in some way that reduces or eliminates the effectiveness of drugs, chemicals, or other agents designed to cure or prevent infections. The bacteria survive and continue to multiply causing more harm. Bacteria can do this through several mechanisms. Some bacteria develop the ability to neutralize the antibiotic before it can do 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, provision of antimicrobial agent that can tackle this resistance problem remain the priority of synthetic chemist [2]. Resistance microbes are increasingly difficult to treat, requiring alternative medication or higher dose. Hence, calls for new antibiotic therapies have been used, but new drug-development is becoming rarer [3]. Cephalosporin are bactericidal and have the same mode of action as other β-lactam antibiotics (such as penicillin), but are less susceptible to β-lactamases. Cephalosporin disrupt the synthesis of the peptidoglycan layer forming the bacterial cell wall. The peptidoglycan layer is important for cell wall structural integrity. The final transpeptidation step in the synthesis of the peptidoglycan is facilitated by trans- peptidases known as penicillin-binding proteins (PBPs) [4]. Resistance to cephalosporin antibiotics can involve either reduced affinity of existing PBP components or the acquisition of a supplementary β-lactam-insensitive PBP. Currently, some Citrobacterfreundii, Enterobacter cloacae, Neisseria gonorrhea, and Escherichia coli strains are resistant to cephalosporin. Some Morganellamorganii, Proteus vulgaris, Providencia rettgeri, Pseudomonas aeruginosa and Serratiamarcescens strains have also developed resistance to cephalosporin to varying degrees [5]. Silver and its compounds have long been used as antimicrobial agents in medicine. 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,

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normally of solids, induced by the input of mechanical energy, such as by grinding in ball mills. It is becoming more intensely studied partly because it can promote reactions between solids quickly and quantitatively, with either no added solvent or only nominal amounts. 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.

2. MATERIALS AND METHODS

All the chemicals used were of analytical grade. These were obtained from Bristol Scientific Company Limited, and used without further purification. The ligand used is cefuroxime (Cfu), while the metals used are copper chloride dihydrate [CuCl2.2H2O] and silver nitrate [AgNO3]. IR spectra of the complexes in KBr pellets were obtained in the range of 4000-400 cm-1 using FTIR spectrometer. Metal analysis was determined by atomic absorption spectroscopy using perkin-Elmer Spectrometer, model 3110. UV-Vis spectra were obtained on UV-2550 Shimazu Spectrophotometer in the wavelength range of 200-800 nm. Synthesis of the Complexes Literature procedure [9] was modified and used for the synthesis of all the metal complexes by mechanochemical method. Cefuroxime (10 mmol, 4.25 g) and copper chloride dihydrate (10 mmol, 1.705 g) were weighed carefully and transferred into a mortar. The two reactants were then crushed (ground) for twenty (20) minutes to obtain homogenous powder. The powder was removed from the mortar and stored in a desiccator. Same procedure was used for silver nitrate (10 mmol, 1.699 g) and cefuroxime (10mmol, 4.25g).

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 The in-vitro antimicrobial activities of the antibiotics and their metal complexes were assayed using disc diffusion method against the following microorganisms; Strepto coccus pneumoniae, Bacillus subtillu, Salmonella typhi, Klebsielia pnuemoniae,

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Escherichia coli, Methicillinresistance staphylococcus aureus (MRSA), Pseudomonas aeruginosa and Staphlylococcus aureus. The suspension of each micro-organism was added to a sterile nutrient agar medium, then spread on the sterile Petri dish plates and allowed to set. Different concentrations (30, 20 and 10) mg/mL of antibiotics and their metal complexes in methanol were placed on the culture media and incubated for 24hrs at 37◦C. Activities were determined by measuring the diameter of the zone of inhibition (mm). The antibiotics and their complexes that showed zone of inhibition of 10 mm and above were further assayed for minimuminhibitory 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.

3. RESULTS AND DISCUSSIONS

The complexes of copper and silver ions obtained are air stable light green and white powders respectively. Both complexes are soluble in polar solvents such as distilled water, methanol, ethanol and dimethylsulfoxide (DMSO). The solubility of the complexes in polar solvents, suggest that the compounds are probably polar. Similar observation was made by [11]. The melting point of the complexes of copper and silver are 110 and 1200C respectively (Table 1). The variation in the melting point of ligand and that of the complexes suggest formation of new compound and also evidence of complexation [12]. The molar conductivity of the complexes falls between 3.6 and 4.5 Scm2 /mol (Table 1). This suggest the complexes are non- electrolytes [13].

Infrared spectra The infrared spectra data of the complexes and its ligand are presented in Table 2. The band assignments are based on comparison with similar studies on mixed ligand complexes and some drug based metal complexes [11]. The vibrations centered around 3190 cm-1 in the free ligand was assigned to v(O-H) stretching frequency, which upon complexation undergo shift in the complexes. The band at 3560 cm-1 in the free ligand was also assigned to v(N-H2) vibration of amine group. While, the band at 1550 cm-1 was assigned to v(C=N) vibration. Similar observation was made by some workers [14].

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The strong intensity band attributed to v(C=O) vibration stretching was observed in the spectra of the free ligand at 1720 cm-1 . The relevant bands were observed in the metal complexes with lower wavelength shift as compared to the ligand couple reduction in their intensities (Table 2). The appearance of new bands at 620 and 630 cm-1 in the spectra of the complexes which is assignable to v(M-O) stretching, suggest formation of the complexes. Electronic spectra The electronic spectral data of the cefuroxime and its complexes are presented in Table 3. Based on previous assignments of related complexes [15-17]. The transition around 349 nm in the spectra of cefuroxime (CFU) was assigned to $\pi \rightarrow \pi^*$ transition (Table 3).Similar observation was made in previous literature [17]. [Cu(CFU)2H2O] complex showed low intensity band at 340nm assigned to MLCT. The [Ag(CFU)NO3] complex, showed absorption band at (287, 301 and 313) nm which indicate a bathochromic shift relative to the free ligand and a weak interaction between the ligand and silver ion which can be assigned 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) 2H2O] and [Ag (L) NO3]. Where L= CFU. Antimicrobial studies Transition metal complexes play a vital role in biological study, some of these have now been widely studied for their antimicrobial and anticancer properties [15] and extensive investigation in the field of metal complexes have been reported [18]. A novel Cu (I) and Ag (I) complexes has also been studied with their antimicrobial activities [16]. In continuation of this discovery, the present study synthesized a new Cu (II) and Ag (I) complexes with cefuroxime using mechanochemical method, and the antimicrobial effects.

International Journal of Chemistry and Pharmaceutical Sciences were observed to see whether the compounds involved in this study exhibit any activity or not. In the present study, both ligand and complexes have been evaluated against both gram positive and negetive bacteria such as: Strepto coccus pneumoniae, Bacillus subtillus, Salmonella typhi, Klebsielia pnuemoniae, Escherichia coli, MRSA, Pseudomonas aeruginosa and Staphlylococcus aureus. The results of the inhibition zones of the selected bacteria due to effect of the ligand and its complexes are presented in Table 5. The results obtained revealed that the complexes were more effective

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against the microorganism than the ligand. The data also showed that Bacillus subtillus was inhibited to the greatest degree by the prepared complexes followed by Staphlyococcus aureus. While, Escherichia coli and Pseudomonas aeruginosa were not inhibited by both the ligand and the complexes at all concentration (Table 5). The complexes also inhibit Klebsielia pnuemoniae at concentration of 20 and 30 mg/mL when compared with the ligand which shows less activity at 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].

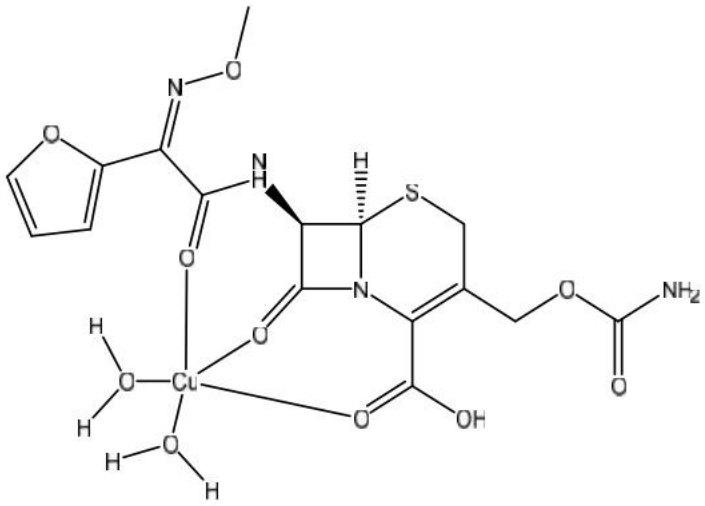

Figure 1: Copper complex of cefuroxime

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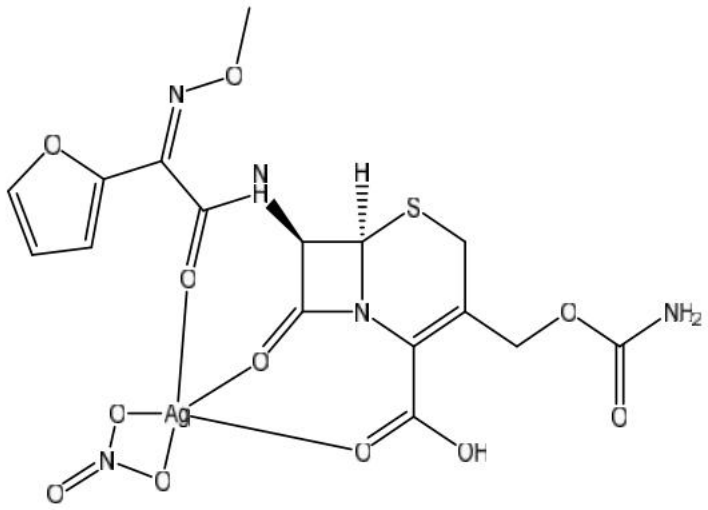

Figure 2: Silver complex of cefuroxime

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	$C_{16}H_{16}N_4O_8S$ (424.39)	White	-	218	-	0.4
[Cu(CFU)2H$_2$O]	[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

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$_2$O]	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

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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
$[Cu(CFU)2H_2O]$	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 pneumonia, B.subtilis=Bacillus subtilis, E.coli= Escherichia coli, S.typhi= Salmonella typhi, K.pneumoniae=Klebsiella pneumonia and P.aruginosa= Psuedomonas aeruginosa.

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

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Compounds	Conc. mg/mL	*MRSA*	*S.aureus*	*B.subtilis*	*S.typhi*	K.*pneum oniae*	*p.aerugi nosa*	*E.coli*	*S.pneu moniae*
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)NO3] 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

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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)N O_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).

4. 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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