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Synthesis, physicochemical and antimicrobial studies of Co(II), Zn(II) and Fe(III) mixed antibiotics metal complexes

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ABSTRACT

Some new transition metal complexes of Co(II), Zn(II) and F(III) with amoxicillin and cephalexin were prepared. The complexes were characterized based on melting point, solubility, conductivity, elemental analysis , IR and UV/Visible spectroscopy. Based on the data obtained, the complexes were proposed to have the formulae [M(A)(B)] where M= Co(II), Zn(II) , Fe(III) ; A= amoxicillin and B = cephalexin. The anti-microbial activities of the complexes / ligands using disc diffusion method was screened against different strains of bacteria; Staphlycoccus aureus, Strep pyogene, Bacillus subtilis, E.coli, Salmonella shigella, Klebsiella pneumonia, Pseudomonas auroginosa .The IR spectra indicates that both ligands A and B coordinated to the metal ion via v(C=O), v(O-H) and v(N-H) respectively. The UV/Visible electronic spectra further revealed that Co(II) and Fe(III) complexes are octahedral while that of Zn(II) is tetrahedral. The complexes melted/decomposed at (200-280)°C. They are soluble in polar organic solvents. Anti-microbial activity studies, revealed that Co(II) complex showed increased activity against the tested organisms while Zn(II) and Fe(III) showed decreased activity as compared with the ligands.

Key words: Synthesis, characterization, antibiotics, metal complexes, anti-microbial

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INTRODUCTION

Antimicrobial agents are among the most commonly used and misused of all drugs [1].The inevitable consequence of the widespread use of antimicrobial agents has been the emergence of antibiotic-resistant pathogens, fuelling an ever increasing need for new drugs [1]. However, the pace of antimicrobial drug development has slowed dramatically, with only a hand full of new agents, few of which are novel, being introduced in to clinical practice each year [1]. Reducing inappropriate antibiotic use is thought to be the best way to control resistant. Resistance to antimicrobial agents may be related to the inability of some of them to reach their sites of action [2]. To enhance or strengthen this phenomenon, there is need to modify or broaden the spectrum of the antimicrobial agents by incorporating them with transition metal ions to form new novel agents (complex compound). Transition metal complexes are cationic, neutral or anionic species in which a transition metal ion is coordinated by ligands [3]. Research has shown significant progress in utilization of transition metal complexes as drugs to treat several human diseases [3]. Many drugs possess modified toxicological and pharmacological properties when they are in form of metal complexes [3]. Mixed ligand or antibiotic metal complexes of transition metals are gaining recognition due to their efficacy against the parent drugs used [4-5]. These complexes show great diversity in action. The use of transition metal complexes as therapeutic compounds has become more and more pronounced. These complexes

offer a great diversity in their action [3]. These results encouraged us to investigate the coordination chemistry of mixed antibiotics metal complexes of amoxicillin and cephalexin with a view to examine their antimicrobial efficacy against the parent drugs.

EXPERIMENTAL SECTION

All the reagents and solvents used were of Analar(AR) grade and were used without further purifications. The melting points of the complexes were determined using Griffin melting point apparatus. The molar conductivity of the complexes in methanol solution (10^{-3}M) at 25^0C was determined using metler P163, while, the electronic absorption spectra of the complexes were obtained using UV-2550 Shimadu Spectrophotometer in the wavelength range of 250-800nm at National Agency for Food, Drug Administration and Control (NAFDAC). Federal Ministry of Science and Technology, Maiduguri, Nigeria. The infrared (IR) spectra were recorded as NaBr disc on Perkin Elmer 1310(IR) at Abubakar Tafawa Balewa University (ATBU) Bauchi, Nigeria. The metal contents of the metal complexes were determined using AA240FS, Fast Sequential Atomic Absorption Spectrometer at multi-user Laboratory, Department of Chemistry, Ahmadu Bello University (ABU) Zaria, Nigeria. Microanalysis of Carbon, Hydrogen and nitrogen (C, H, N) was performed using Perking Elmer model 2400 series 11CHN S/O Element analyzer in South Africa. The antibacterial activity was determined using disc diffusion method at Faculty of Veterinary Medicine, University of Maiduguri, Nigeria.

Synthesis of the complexes

The complexes were prepared using a literature procedure [6]. The antibiotics were labelled as A and B for amoxicillin trihydrate and cephalexin monohydrate respectively. Aqueous (20ml) solution of the antibiotics, [10mmol, 4.196g of A and 10mmol, 3.834g of B] in separate conical flask was heated on a water bath until a clear solution was obtained. They were then mixed together, and the solution of the mixed antibiotics was further mixed with the aqueous (20ml) solution of the metal (II) salts [10mmol, 2.379g of $CoCl_2.6H_2O$, 10mmol, 2.975g of $Zn(NO_3)_2.6H_2O$ and 10mmol of 2.235g of $FeCl_3.6H_2O$] respectively in 1:1:1 mole ratio. The mixture was refluxed for 4hrs on a hot plate magnetic stirrer, after which the volume of the solution was concentrated to half of the initial volume. The final product obtained was allowed to cool, washed with water, diethyl ether and then dried in a vacuum over $CaCl_2$.

Antimicrobial activity

The invitro antimicrobial properties of the antibiotics and their metal complexes were assayed using the following Gram positive bacteria: *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis* and Gram negative: *Salmonella typhi, Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa*, using disc diffusion method in methanol [7].The suspension of each micro-organism was added to a sterile agar medium (nutrient agar), then poured in a sterile petri plate and left for solidification. Different concentrations (30,20 and 10)µg/ml of the 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).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the antibiotics and their complexes

The antibiotics and their complexes that showed zone of inhibition of 10mm and above, were further assayed for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using concentrations of (2, 4 and 6)µg/ml in methanol with the same bacterial species as describe above.

RESULTS AND DISCUSSION

The analytical data and some physical properties of the ligands and their metal complexes are presented in Table 1. The ligands (A and B) on interaction with Co(II), Zn(II) and Fe(III) formed complexes with moderate yield ranging from 35-94%. The complexes showed various colours from green to brown and yellow. This is typical of transition metal complexes. The complexes obtained are non-hygroscopic, air and photo stable, crystalline powder with different melting point ranging from (200 – 250)°C. The complex of Co(II) decomposed while, Zn(II) and Fe(III) complexes gave sharp melting points, this suggest they may be probably pure [7]. The melting points of the complexes are higher than their respective antibiotics; this suggests the formation of new product [8]. The lower value of molar conductivity (8.6 – 14.1 x 10^{-3} Scm^2mol) indicates the non-electrolytic behaviour of these complexes Table1 [9]. The solubility of complexes and their ligands was determined at room temperature (37°C) in polar and

non-polar solvents such as; distil water, methanol, ethanol, chloroform, acetone, benzene and pet.ether, and then warmed over water bath for the complexes that are insoluble in cold solvents. They were all found to be either soluble or slightly soluble solvents like distil water, methanol and ethanol, which indicates they may be polar.

Table 1: Physical characteristics for the antibiotics and their metal (II) complexes

Compounds	Molecular formula (Molar mass)	Colour	Melting point ^{O}C	Yield (g) (%)	Molar Conductivity ($Scm^{-2}mol^{-1}$)
A	$C_{16}H_{19}N_3O_4S.3H_2O$ (419.45)	White	194	_	4.2×10^{-3}
B	$C_{16}H_{17}N_3O_4S.H_2O$ (365.41)	White	195	_	7.5×10^{-3}
[Co(A)(B)]	$Co(C_{32}H_{34}N_6O_8S_2)$ (752.93)	Green	220 – 250	5.70 (70)	8.6×10^{-3}
[Fe(A)(B)]	$Fe(C_{32}H_{34}N_6O_8S_2)$ (749.85)	Brown	200	3.0 (40)	14.1×10^{-3}
[Zn(A)(B)]	$Zn(C_{32}H_{34}N_6O_8S_2)$ (759.37)	Yellow	230	9.0 (94)	9.5×10^{-3}

Table 2: The microanalysis and metal estimation data

Compounds	Molecular formula (Molar mass)	Microanalysis: found (calculated) %			
		C	H	N	M
[Co(A)(B)]	$Co(C_{32}H_{34}N_6O_8S_2)$ (752.93)	49.07 (50.98)	4.17 (4.68)	11.07 (11.00)	7.76 (7.81)
[Fe(A)(B)]	$Fe(C_{32}H_{34}N_6O_8S_2)$ (749.86)	50.54 (51.00)	4.46 (4.70)	11.46 (11.15)	7.45 (7.43)
[Zn(A)(B)]	$Zn(C_{32}H_{34}N_6O_8S_2)$ (759.37)	50.38 (50.55)	4.76 (4.64)	11.12 (11.05)	8.60 (8.59)

Microanalysis

Microanalysis and metal estimation data of the complexes are presented in Table 2. From the results obtained, the percentage of C, H and N are in good agreement with those of the proposed structures. The metal ion percentage also agrees with the proposed structure. Based on the data obtained, the compounds analysed as [M(A)(B)] (Figure 1and 2).

Infrared spectra

The selected vibrational frequencies for the antibiotics and their metal complexes are presented in Table 3. The appearance of medium and weak bands at $3560cm^{-1}$ in the spectra of the free ligands A and B was assigned to v(O-H) bond stretching [10].The strong and medium bands at $3250cm^{-1}$ and $3300cm^{-1}$ respectively in the spectra of the free ligands, is due to v_{asy} (NH) and v_{sy}(NH) stretching of secondary amide [10]. The strong and medium bands in the ligands at $1750cm^{-1}$ and $1780cm^{-1}$ is due to v(C=O) stretching [11]. However, all bands were decreased either by 5,10 or $15cm^{-1}$ in the spectra of the ligands. This suggests the involvement of v(NH), v(O-H) and v(C=O) as sites of coordination to the metal ion in the complexes. According to some literature reports [12] , coordination of NH results in splitting or shifting of bands to lower frequency side or decrease in intensity. In the present study, such changes were observed in the spectra of the complexes which further affirm the involvement of v(NH) as coordination site. On complexation, v(O-H) appeared at $3520cm^{-1}$,$3550cm^{-1}$ and $3540cm^{-1}$ in the spectra of the three complexes. The shift in the frequency of absorption to lower energy, suggests the deprotonation of v(COOH) and hence the bonding is through the oxygen atom of the carboxylate (COO) ion [13]. The v(C=O) stretching decreased to $1650cm^{-1}$,$1700cm^{-1}$ and $1600cm^{-1}$ in the spectra of the complexes. This indicates the involvement of the carbonyl oxygen in the coordination [14].

Table 3: **Relevant infrared frequencies (cm^{-1}) of the antibiotics and their metal complexes**

Compounds	Infrared frequencies (cm^{-1})							
	v(N-H)	v(O-H)	v(C=O)	v(COO)	v(C-N)	v(NH_2)	v(M-N)	v(M-O)
A	3250s	3560m	1750s	1560m	1440m	2900s	-	-
B	3300m	3560w	1780m	1530w	1450w	3000w	-	-
[Co(A)(B)]	3240s	3520w	1650w	1480m	-	2900m	-	640w
[Fe(A)(B)]	3280w	3550s	1700s	1520m	1420w	-	-	560w
[Zn(A)(B)]	3200b	3540w	1600b	1450m	-	2900m	-	670w

Note: vs = very strong, s = strong, b = broad, m = medium, w = weak,

Table 4: Electronic spectral data for the antibiotics and their metal complexes

Compounds	Absorption(cm^{-1})	Band assignment	Octahedral
A	30461	$n \rightarrow \pi^*$	–
	42633	$n \rightarrow \pi^*$	
B	36429	$n \rightarrow \pi^*$	–
	42627	$n \rightarrow \pi^*$	
[Co(A)(B)]	20000	$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$	Octahedral
	16529	$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$	
	15385	$^4T_{2g}(F) \rightarrow {}^4A_{2g}$	
[Fe(A)(B)]	23256	MLCT	Octahedral
	20000	$^2T_{2g}(F) \rightarrow {}^5E_g$	
	14286	$^2T_{2g}(F) \rightarrow {}^5E$	
[Zn(A)(B)]	28339	MLCT	Tetrahedral
	33093	MLCT	

Electronic spectra

The UV/Vis spectra of Co(II), Fe(III) and Zn(II) complexes with the ligands were recorded at 190 – 800nm using (10^{-3})molar solution in methanol. From the results obtained in Table 4, the ligand A showed two distinct peaks at 30461cm^{-1} and 42633cm^{-1} which are assignable to $n \rightarrow \pi^*$ [15]. The ligand B also showed two distinct peaks at 36429cm^{-1} and 42627cm^{-1} assignable to $n \rightarrow \pi^*$[15]. The electronic spectra of Co(II) complex exhibited three peaks in the visible region at 20000, 16529 and 15385cm^{-1} which are assignable to $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{2g}(F) \rightarrow {}^4A_{2g}$ transitions respectively in octahedral geometry [17- 18]. The Fe(III) complex, showed an absorption band at 23256cm^{-1} which is due to charge transfer [14]. Two other bands appeared at 20000 and 14286 cm^{-1} assignable to $^2T_{2g}(F) \rightarrow {}^5E_g$ which suggests an octahedral geometry [13]. In the spectra of the Zn(II) complex, two well defined bands appeared in the UV region at 28339 and 33093cm^{-1}. These may be assigned to charge transfer trasitions [13] . This compound did not show any band in the visible region. This could be attributed to the filled d^{10} configuration of the metal ion, where d-d transitions are not possible (Table 4) .

Antibacterial activity

The antibacterial activity of the complexes were assayed using the following bacterial species which includes Gram positive; *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis* and Gram negative: *Salmonella typhi, Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa*. This was done using disc diffusion method in methanol [7,16]. The concentrations of (10, 20 and 30µg/ml) for both the ligands and their complexes were used. The results shows that complexes of Co(II) and Fe(III) showed increased activity when compared with the parent drugs while, Zn(II) complex showed decreased activity (Table 5).

Table 5: Antimicrobial activities of the antibiotics and their metal complexes

Cpds	Conc. µg/ml	*S.aureus* (mm)	*S.pyogene* (mm)	*B.subtillis* (mm)	*E.coli* (mm)	*Salmonella* (mm)	*K.pnuemoni* (mm)	*P.aeruginosa* (mm)
A	10	10±0.07	17±0.08	9±0.02	0.000	0.000	15±0.23	0.000
	20	15±0.01	20±0.09	12±0.27	0.000	0.000	18±026	0.000
	30	17±0.03	23±0.90	14±0.07	8±1.01	0.000	20±033	0.000
B	10	12±0.13	15±1.00	7±0.01	9±0.10	12±0.97	7±0.09	8±0.98
	20	18±0.17	18±0.08	9±0.45	12±0.1	15±0.49	9±0.78	13±0.54
	30	25±0.20	22±0.22	12±1.02	15±0.6	17±0.65	11±0.98	15±0.79
[Co(A)(B)]	10	20±0.03	18±0.06	16±0.09	13±0.6	15±0.07	10±0.03	10±0.03
	20	22±0.04	23±0.01	20±0.04	20±0.9	18±0.04	15±1.00	18±0.19
	30	42±0.08	30±0.06	28±0.05	25±0.1	29±0.48	25±0.06	25±0.33
[Fe(A)(B)]	10	20±0.07	17±0.09	20±0.04	10±0.4	5±0.01	0.000	7±0.67
	20	30±0.30	25±0.07	20±0.09	10±0.8	12±0.94	15±2.09	15±0.98
	30	30±0.17	28±0.33	20±1.20	20±0.1	18±0.39	25±1.06	18±0.57
[Zn(A)(B)]	10	08±0.13	10±.030	0.000	0.000	0.000	0.000	0.000
	20	10±0.26	15±0.27	0.000	0.000	0.000	0.000	0.000
	30	13±0.23	18±0.20	0.000	0.000	0.000	0.000	0.000

Table 6: The minimum inhibitory concentration (MIC) of the antibiotics and their metal complexes

Compounds	*Concentration µg/ml*	*Staph.aureus*	*Strep.pyogene*	*Bacillus subtilis*	*K. pneumonia*
A	2	-	-	-	-
	4	-	-	-	-
	6	+	+	+	-
B	2	-	-	-	-
	4	-	-	-	-
	6	+	+	+	-
[Co(A)(B)]	2	-	-	-	-
	4	-	-	-	-
	6	+	+	+	+
[Fe(A)(B)]	2	-	-	-	-
	4	-	-	-	-
	6	+	+	+	+
[Zn(A)(B)]	2	-	-	-	-
	4	-	-	-	-
	6	-	-	+	-

Table 7: The minimum bactericidal concentration (MBC) of the antibiotics and their metal complexes

Compounds	*Concentration µg/ml*	*Staph.aureus*	*Strep.pyogene*	*Bacillus subtilis*	*K. pneumonia*
A	4	-	-	-	-
	6	-	-	-	-
	8	+	+	+	-
B	4	-	-	-	-
	6	-	-	-	-
	8	+	+	+	+
[Co(A)(B)]	4	-	-	-	-
	6	-	-	-	-
	8	+	+	+	+
[Fe(A)(B)]	4	-	-	-	-
	6	-	-	-	-
	8	+	+	+	+
[Zn(A)(B)]	4	-	-	-	-
	6	-	-	-	-
	8	+	+	-	-

The study on the minimum inhibitory concentration (MIC), and minimum bacterial concentration (MBC) of both the antibiotics and their metal complexes are presented in Tables 6 and 7. The results showed that, the antibiotic A (amoxicillin) have maximum MIC value of 6µg/ml on *Staphylococcus aureus*, *Streptococcus pyogenes*, *and Bacillus Subtilis* respectively. The antibiotic B (cephalexin) shows maximum MIC value of 6µg/ml against *Staphylococcus aureus* and *Streptococcus pyogenes* only (Table 6). The metal complexes: [Co(A)(B)] showed activity at all the concentrations on the tested organism. [Fe(A)(B)] showed activity on all the bacteria tested at concentration of 6µg/ml.

The results of minimum bactericidal concentration revealed that, only [Co(A)(B)] and [Fe(A)(B)] showed increased activity as compared with the parent drugs at various concentrations tested (Table 7).

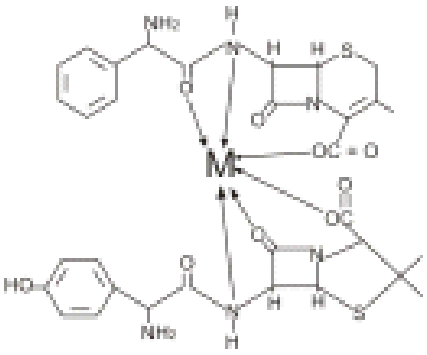

Fig 1; Proposed structure of the metal complexes, where M = Co(II) and Fe(III)

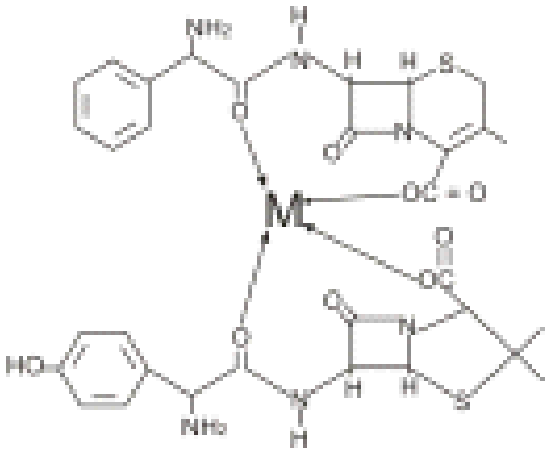

Fig 2: Proposed structure of the metal complex, where M = Zn(II)

CONCLUSION

Based on the analytical data obtained , both ligands coordinated to the metal ions through v(C=O), v(N-H) and v(COO) respectively due to their structural similarity. The proposed geometry of Co(II) and Fe(III) are octahedral while Zn(II) is tetrahedral. The complexes of Co(II) and Fe(III) showed increased antimicrobial activity when compared with parent drugs while Zn(II) showed decreased activity. The elemental percentages are in agreement with those of proposed structures. Molar conductivity results in methanol have revealed that the complexes are non-electrolytes.

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