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Synthesis, Spectral Characterization and Antimicrobial Activity of Some Metal Complexes of Mixed Antibiotics

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Abstract Mixture of cephalexin (Cep) and amoxicillin (amx) in 1:1 mole ratio, interact with transition metal ions to give $[M(cep)(amx)].3H_2O$ complexes in 1:1:1 mole ratio, where M = Co(II), Ni(II), Zn(II) and Fe(III). The complexes were characterized by physicochemical and spectroscopic analysis. The UV/Vis spectral data suggestedan octahedral geometry for Co(II), Ni(II) and Fe(III) structures, while Zn(II) complex adopted tetrahedral geometry. The IR spectral data shows that the ligands coordinated to the metal ion through their v(NH), v(COO) and v(C=O) functional respectively due to the similarity in their structures. The complexes have been screened for antimicrobial activity against different strains of bacteria which includes Gram positive, Gram negetive and fungus, and the results are compared with the activity of the ligands (antibiotics).

Keywords Antibiotic, Antibiotic resistance, Bacteria, Complexes, Transition metals

Introduction

Antibiotic resistance is said to occur when bacteria under goes transformation in such a manner that it can easily weaken or render antimicrobial agent ineffective [1]. 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. Antibiotics kill or inhibit the growth of susceptible bacteria [1]. Sometimes one of the bacteria survives because it has the ability to neutralize or escape the effect of the antibiotic; that one bacterium can then multiply and replace all the bacteria that were killed off [1]. Exposure to antibiotics therefore provides selective pressure, which makes the surviving bacteria more likely to be resistant. In addition, bacteria that were at one time susceptible to an antibiotic can acquire resistance through mutation of their genetic material or by acquiring pieces of DNA that code for the resistance properties from other bacteria. The DNA that codes for resistance can be grouped in a single easily transferable package. This means that bacteria can become resistant to many antimicrobial agents because of the transfer piece of DNA. The of problem bacteria resistance to antibiotic or antimicrobial agents is increasing at alarming rate globally [2]. This problem is attributed to the wider use of antibiotics in humans and animals, and in the areas other than treatment and prophylaxis of diseases [3]. In view of these, different approaches and strategies have been adopted in order to find alternative to the problem of bacteria resistance to antibiotics. These includes, modifying the activities of the antibiotic or broaden their spectrum

to be active against both Gram positive and Gram negative bacteria. Among the various models used to enhance the efficacy of antibiotics or antimicrobial agent, use of transition metals which includes both first and second rows, produces compounds with promising antimicrobial activity when compare with parent drug used. In our earlier work, we reported synthesis of transition metal complexes with mixed antibiotics. The compounds demonstrate high antimicrobial activity against microorganism tested as compared to the parent drugs [4-5]. In continuation of our work, different antibiotic were used with metals in order to evaluate the role of the metals on different antibiotics.

Experimental

Material and Instruments

All the reagents and solvents used were of analytical grade and were used without further purification. The melting point of the complexes were determined using Griffin melting point apparatus. Molar conductivity measurement (10^{-3}M solution in methanol) was obtained on the metler P163, while elemental analysis were carried out on a perking-Elmer model 2400 series 11CHNS/O elemental analyzer. The metal content of the complexes was determined using AA240FS, Fast Sequential Atomic Absorption Spectrometer. The electronic absorption spectra of the complexes were obtained using UV-250 Shimadu Spectrophotometer in the wavelength range of 250-800 nm. The infrared (IR) spectra were recorded as NaBr disc on Perking Elmer 1310(R) in the range of 4000-400 cm^{-1}. The antibacterial activity was determined using disc diffusion method.

Synthesis of the Complexes

The method described by Ogunniran et al., [6] was modified and adopted for the synthesis of all the complexes. Aqueous (20 mL) solutions of the antibiotics, [10 mmol, 3.834 g of Cephalexin monohydrate (Cep) and 10 mmol, 4.196 g of Amoxicillin trihydrate (Amx)] were mixed in 1:1 mole ratio. The solution of mixed antibiotics was further mixed with the aqueous (20 mL) solution of [10 mmol, 2.380 g of $CoCl_2.6H_2O$, 2.376 g of $NiCl_2.6H_2O$, 2.235 g of $Zn(NO_3)_2$ and 2.235 g of $FeCl_3.6H_2O$]in 1:1:1 mole ratio. The reaction mixture was refluxed for 4 hrs on a hot plate magnetic stirrer 50°C. The volume of the solution was concentrated to half of the initial volume. The product obtained was allowed to cool, washed with water, diethyl ether and then dried in a vacuum over $CaCl_2$.

Antimicrobial Screening

The *in vitro* antimicrobial properties of the antibiotics and their metal complexes were assayed using disc diffusion method against the following bacteria species; *Staphylococcusaureus, Streptococcus pyogenes, Bacillus subtilis, Salmonella typhi, Escherichia coli, Klebsiellapnuemonia, Psuedomonasaeruginosa* and *Candida albicans*. The suspension of each micro-organism was added to a sterile nutrient agar medium, then spread on the sterile petri plates and allowed to set. Different concentrations (30, 20 and 10) µg/ml of antibiotics and their metal complexes in methanol were placed on the culture media and incubated for 24 hrs at 37 ◦C. Activities were determined by measuring the diameter of the zone showing complete inhibition (mm). The antibiotics and their complexes that showed 10mm zone of inhibition were further assayed for minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) using samples concentration of (6, 4 and 2) µg/ml in methanol using same bacterial species in peptone water.

Results and Discussion

The metals Co(II), Ni(II), Fe(III) and Zn(II) complexes of cephalexin (Cep) and Amoxicillin (Amx) were synthesized by reaction of metal salts with mixture of cephalexin and amoxicillin. The complexes were obtained in moderate yield ranging from 36-56 % (Table).The complexes were characterized by AAS, Conductivity, infrared, UV-Visible and microanalysis. All the complexes are air stable and soluble in methanol and ethanol except Zn(II) complex which is slightly soluble in both methanol and ethanol. They are insoluble in non-polar organic solvents. The physical properties of the complexes are shown in Table 1. All the complexes synthesized are coloured ranging from white to green, yellow and brown. This is typical of transition metal complexes. The complexes are also non-hygroscopic solids with different melting point ranging from 210-240 °C. All the complexes have melting point higher than their parent drug probably due to complexation [7].

Table 1: Physical characteristics of the ligands and their metal (II) complexes

Compounds	Molecular formula (Molar mass)	Colour	M.pt/d(°C)	Yield (g) (%)	Molar conductivity Scm^2/mol
Cep.	$C_{16}H_{17}N_3O_4S.\ H_2O$ (365.41)	White	195	_	$7.5x10^{-3}$
Amx.	$C_{16}H_{17}N_3O_4S.\ 3H_2O$ (419.45)	White	194	_	$4.2x10^{-3}$
$[Co(Cep.)(Amx.)]\cdot 3H_2O$	$Co(C_{32}H_{40}N_6O_{11}S_2)$ (806.93)	Yellow	230	5.67 (36)	$12.4x10^{-3}$
$[Ni(Cep.)(Amx.)]\cdot 3H_2O$	$Ni(C_{32}H_{40}N_6O_{11}S_2)$ (806.71)	Green	225d	4.0 (48)	$10.0x10^{-3}$
$[Fe(Cep.)(Amx.)]\cdot 3H_2O$	$Fe(C_{32}H_{40}N_6O_{11}S_2)$ (803.86)	Brown	210	3.5 (40)	$10.8x10^{-3}$
$[Zn(Cep.)(Amx.)]\cdot 3H_2O$	$Zn(C_{32}H_{40}N_6O_{11}S_2)$ (813.37)	Yellow	240	4.8 (56)	$18.7x10^{-3}$

Cep. = Cephalexin, Amx. = Amoxicillin, d = decompose

Table 2: The microanalysis and metal estimation data of the complexes

Compounds	Molecular formula (Molar mass)	Microanalysis: found (calculated) %			
		C	H	N	M
$[Co(Cep.)(Amx.)]\cdot 3H_2O$	$Co(C_{32}H_{40}N_6O_{11}S_2)$ (806.93)	47.37 (47.59)	5.35 (5.00)	9.37 (9.87)	7.69 (7.83)
$[Ni(Cep.)(Amx.)]\cdot 3H_2O$	$Ni(C_{32}H_{40}N_6O_{11}S_2)$ (806.71)	48.00 (47.60)	5.00 (4.96)	10.82 (10.41)	7.75 (7.80)
$[Fe(Cep.)(Amx.)]\cdot 3H_2O$	$Fe(C_{32}H_{40}N_6O_{11}S_2)$ (803.86)	50.43 (51.26)	4.47 (4.44)	8.37 (8.22)	7.46 (7.45)
$[Zn(Cep.)(Amx.)]\cdot 3H_2O$	$Zn(C_{32}H_{40}N_6O_{11}S_2)$ (813.37)	53.25 (53.17)	5.51 (5.52)	10.24 (10.07)	8.70 (8.60)

Microanalysis

The result of microanalysis of the metal (II) complexes is presented in Table 2. From the result obtained, the %C, H and N are conformity with the proposed structures. Based on the evaluation of the microanalysis data, it shows that the compounds analyzed as $[M(Cep)(Amx)]\cdot 3H_2O$ where M = Co^{II}, Ni^{II}, Fe^{III}, and Zn^{II}. While, Cepand Amx represent cephalexin and amoxicillin respectively. The percentage of metal ion also agrees with the proposed structures (Table 2).

Infrared spectra

The IR spectral data of the free ligand and the metal complexes are presented in Table 3. The band assignments were done by comparing the spectra of the free ligand with metal complexes and also related compounds in the literature [8-10].

Table 3: Relevant Vibrational Bands for the Antibiotics and Their Metal Complexes

Compounds	V(N-H)	V(O-H)	V(C=O)	V(COO)	V(C-N)	$V(NH_2)$	M-O
Cep.	3060s	3560w	1780m	1530w	1450w	3000w	-
Amx.	3020s	3500w	1760s	1540m	1400m	2900s	-
$[Co(Cep.)(Amx.)]\cdot 3H_2O$	3000s	3300b	1680m	1490b	1400w	2945s	670w
$[Ni(Cep.)(Amx.)]\cdot 3H_2O$	3380w	3380s	1700s	1620m	1420w	-	650w
$[Fe(Cep.)(Amx.)]\cdot 3H_2O$	3020s	3100b	1630m	1430m	-	-	660w
$[Zn(Cep.)(Amx.)]\cdot 3H_2O$	2980s	3200b	1660b	1580m	-	-	490w

Note; b = broad, m= medium, s = strong, sh = sharp, w = weak

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

Compounds	λ_{max}(cm^{-1})	ε_{max} ($mol^{-1}cm^{-1}$)	Band assignment	Geometry
Cep	36429, 426270	-	n $\rightarrow\pi^*$	
Amx	30461, 42633	-	n $\rightarrow\pi^*$	
[Co(Cep)(Amx)]·$3H_2O$	20000	193140	$^4T_{1g}$ (F) $\rightarrow$ $^4T_{1g}$ (P)	Octahedral
	17241		$^4T_{1g}$ (F) $\rightarrow$ $^4A_{2g}$ (F)	
	15385		$^4T_{2g}$ (F) $\rightarrow$ $^4A_{2g}$	
[Ni(Cep)(Amx)]·$3H_2O$	19231	192800	$^3A_{2g}$ (F) $\rightarrow$ $^3T_{2g}$ (F)	Octahedral
	15385		$^3A_{2g}$ (F) $\rightarrow$ $^3T_{1g}$ (P)	
[Fe(Cep)(Amx)]·$3H_2O$	21053	5648900	MLCT	Octahedral
	15385		$^2T_{2g}$(F) $\rightarrow$ ^5E_g	
	14286		$^2T_{2g}$(F) $\rightarrow$ ^5E_g	
[Zn(cep)(Amx)]·$3H_2O$	18182	2219000	CT	Tetrahedral
	15385		CT	

The strong band at 3020 and 3060 cm^{-1} in the free ligand which shifted to 2980-3380 cm^{-1} in the complexes is assigned to v(N-H) stretching frequency. The v(COO) mode absorbed as weak and medium in the region of 1530-1540 cm^{-1} in the ligand and shifted to the region of 1430-1620 cm^{-1} in the complexes. The shift to higher wave numbers on complexation could be due to a change in the orientation of the v(COO) bond with respect to hydrogen in the ligand and complexes. This observation is in agreement with similar observation by in the literature [11-13]. The bands at the region 1760-1780 cm^{-1} in the free ligands, which shifted to the region of 1630-1700 cm^{-1} in the complexes are assigned to v(COO) stretching mode. The decrease in the wave number in the complexes, suggest complexation through carbonyl functional group to the metal ion. Similar observation was made by [14].

Electronic Spectra

The electronic spectral data of the ligands and their complexes are presented in Table 4. The ligand Cep showed to distinct bands at 36429 and 426270 cm^{-1}, while Amx showed two bands at 30461 and 42633 cm^{-1}. These could be assigned n$\rightarrow \pi^*$ transition in the freeligands [15]. Co(II) complex showed three bands at 2000, 17241 and 15385cm^{-1} , respectively, which could be assigned to $^1A_{1g} \rightarrow {}^1T_{1g}$ transition of an octahedral geometry [16-17]. The Ni(II) complex gave two bands at 19231 and 15385 cm^{-1} , which corresponds to $^3A_{2g} \rightarrow {}^3T_{1g}$ and $^3A_{2g} \rightarrow {}^3T_{2g}$. the Zn(II) complex with no d-d transition shows two bands at 18182 and 15385 cm^{-1} assigned to MLCT in tetrahedral geometry.[18-19]Fe(III) complex showed three bands at 21053, 15385 and 14286cm^{-1} assignable to $^5T_{2g}(F) \rightarrow {}^5E_g$ transition. This transition is typical of octahedral geometry.

Antimicrobial Screening

The result of the antimicrobial screening of complexes and their ligands against both Gram positive and Gram negative bacteria using different concentration is presented in Table 5.

Table 5: Antimicrobial activities of ligands and their metal complexes

Compound	Conc. µg/g	*S. aureus*	*S. pyogenes*	*B. subtilis*	*E. coli*	*S. typhi*	*K. pneumoniae*	*P. aeruginosa*	*C. albicans*
Cep.	10	+	++	-	+	+	-	+	+
	20	++	++	-	+	+	-	+	+
	30	+++	++	++	+	++	-	++	++
Amx.	10	+	-	+	+	-	+	-	+
	20	++	-	+	+	-	+	-	-
	30	++	-	+	++	-	+	-	-
[Co(Cep.)(Amx.)]·$3H_2O$	10	+	+	++	++	-	++	++	-

	20	++	+	++	++	-	+++	++	-
	30	+++	+	+++	++	-	+++	+++	-
$[Ni(Cep.)(Amx.)]\cdot 3H_2O$	10	++	-	-	+	-	+	+	+
	20	+++	+	-	+	++	+++	+	+
	30	+++	++	++	++	+++	++++	++	+
$[Fe(Cep.)(Amx.)]\cdot 3H_2O$	10	-	-	-	-	-	-	-	-
	20	-	-	-	-	-	-	-	+
	30	-	-	-	-	-	-	-	+
$[Zn(Cep.)(Amx.)]\cdot 3H_2O$	10	-	+	-	-	+	-	-	-
	20	-	+	-	-	+	-	-	-
	30	+	+	-	-	+	-	-	-

s.aureus = staphylococcus aureus, s.pneumoniae = Streptococcuspneumonia,B.subtilis=Bacillussubtilis, E.coli= Escherichia coli, S.typhi= Salmonella typhi, K.pneumoniae=Klebsiella pneumonia and P.aruginosa= Psuedomonasaeruginosa.

(-) = 0 – 5 ± 0.15mm = resistant,(+) = 5 -10 ± 0.07mm = slightly susceptible

(++) = 10 – 15 ± 0.33mm = susceptible, (+++) = 15 – 45 ± 1.20mm = highly susceptible

The result of the antimicrobial activity reveal that only complexes of Co(II) and Ni(II) shows increased activity on some microorganism when compared with the ligands (Table 5). The complexes of Co(II) and Ni(II) were further subjected to MIC and MBC. The result showed that boththe complexes and their ligands (antibiotics) had MIC and MBC value 6µg/g on some microorganism tested (Table 6 and 7).

Table 6: Minimum inhibitory concentration (MIC) of the ligands with some their complexes

Compound	**Conc. µg/g**	***S. aureus***	***S. pyogenes***	***B. subtilis***	***E. coli***	***S. typhi***	***K. pneumoniae***	***P. aeruginosa***	***C. albicans***
Cep	2	R	R	R	R	R	R	R	R
	4	R	R	R	R	R	R	R	R
	6	S	S	S	S	R	R	R	R
	2	R	R	R	R	R	R	R	R
Amx	4	R	R	R	R	R	R	R	R
	6	S	S	S	S	S	S	R	R
$[Co(Cep)(Amx)]\cdot 3H_2O$	2	R	R	R	R	R	R	R	R
	4	R	R	R	R	R	R	R	R
	6	S	S	R	S	S	S	S	S
$[Ni(Cep)(Amx)]\cdot 3H_2O$	2	R	R	R	R	R	R	R	R
	4	R	R	R	R	R	R	R	R
	6	S	S	S	R	S	S	S	S

R = resistant and S = susceptible

Table 7: Minimum bactericidal concentration of the ligands with some of their complexes

Compound	**Conc. µg/g**	***S. aureu***	***S. pyogenes***	***B. subtilis***	***E. coli***	***S. typhi***	***K. pneumonie***	***P. aeruginosa***	***C. albicans***
Cep	6	S	R	S	R	R	R	R	R
Amx	6	S	S	S	S	S	R	R	R
$[Co(Cep)(Amx)]\cdot 3H_2($	6	S	S	S	S	S	R	S	S
$[Ni(Cep)(Amx)]\cdot 3H_2O$	6	S	S	S	S	S	S	S	S

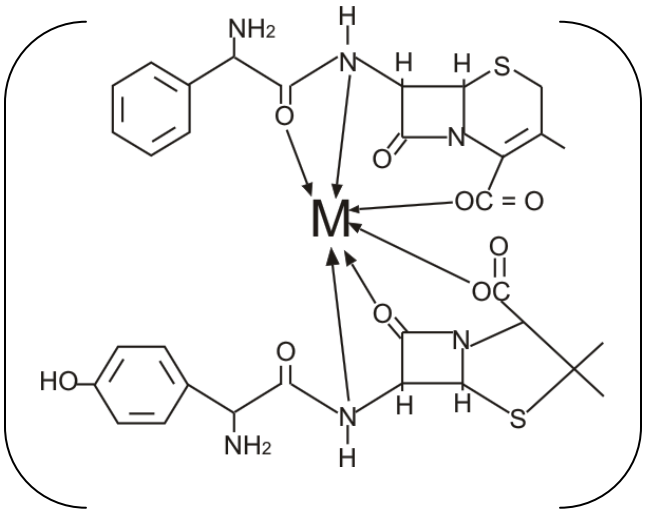

Figure 1: Proposed structure of the metal complexes, where M = Co(II), Ni(II) and Fe(III)

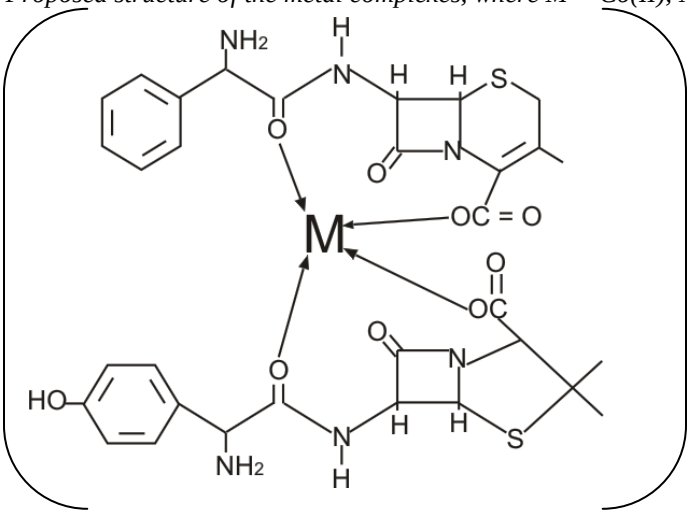

Figure 2: Proposed structure of Zn(II) complex, where M= Zn(II)

Conclusion

¶ Based on the data obtained, both the ligands coordinated to the metal ions through v(NH), v(C=O) and v(COO) due to their structural similarities. The proposed structure for Co(II), Ni(II) and Fe(III) is octahedral, while Zn(II) complex is tetrahedral. All the complexes have three water molecules outside their coordination sphere. The antimicrobial result, showed increase activity for Co(II) and Ni(II) complexes. Decrease activity was observe against Fe(III) and Zn(II) when compared with the parent drugs. The elemental percentages are also in good agreement with the proposed structure.

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