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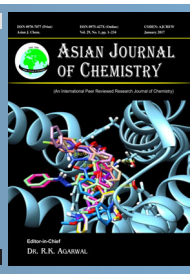

Synthesis, Characterization and Antimicrobial Studies of Coordination Compounds of L-Serine and their Mixed Ligand Complexes with L-Aspartic Acid

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The coordination complexes of Co(II), Ni(II) and Cu(II) with L-serine and its mixed ligand with L-aspartic acid were synthesized and characterized. The compounds were characterized using UV-visible, infrared spectra and metal analyses. Antimicrobial study of the complexes was carried out, the tested microbes included two Gram-positive and five Gram-negative bacteria and one fungus. The results suggested an octahedral geometry for the complexes with L-aspartic acid and L-serine acting as tridentate ligands. The ligands coordinated with the metal ions through N and O donor atoms respectively. The antimicrobial study showed the complexes possess activity against the micro-organisms.

Keywords: L-Serine, L-Aspartic acid, Mixed ligand complexes, Antimicrobial activity, Metal(II) ion.

INTRODUCTION

Mixed ligand complexes of transition metals with various amino acids have been studied and possess antimicrobial, antifungal and cytotoxic activities [1]. Research has shown significant progress in utilization of transition metal complexes as drugs to treat several human diseases like carcinomas, diabetes and neurological disorder [2]. They have also found application for infection control and as anti-inflammatory agents. Mixed ligand amino acids complexes are of relevance as models for enzyme inhibition [3,4]. The geometry, number of ligands, donor atoms and nature of binding of these ligands is key to understanding specific physiological functions [5]. Some mixed ligand amino acid complexes have exhibited enhanced antimicrobial activity compared to the parent complexes [6,7]. Copper(II) complexes of the type $[M(Q)(L)]\cdot 2H_2O$ have been synthesized using 8-hydroxylquinoline (HQ) as a primary ligand and N-and O-donor amino acids (HL) such as L-thronine, L-proline, L-hydroxylproline, L-isoleucine and L-serine as secondary ligands. The metal complexes were characterized using elemental analysis, electrical conductivity, room temperature magnetic susceptibility measurements, spectral and thermal studies [8]. Aspartic acid and serine are both capable of binding as tri-, mono- and bi-dentate ligands. As a tridentate ligand aspartic acid forms 6 and 5 member chelates, on the other hand serine forms five member chelates. Such possibilities involving their coordination behaviour is generally of interest to coordination chemists. Therefore, in this work, we report the syntheses, characterization and antimicrobial activity of mixed ligand complexes with L-serine (L) and L-aspartic acid (L') acting as tridentate ligands.

EXPERIMENTAL

Chemicals reagents were purchased from Sigma Aldrich (USA). All the starting materials were of analytical grade and were used without further purification.

Melting points were recorded by using Gallenkamp (Variable heater) melting point apparatus. Infrared spectra were recorded using FTIR-8400 Shimadzu as KBr disk in the range 4000-400 cm^{-1}. UV-visible spectra were measured using Shimadzu UV-160A UV spectrophotometer at room temperature. The percent metal content of the complexes were determined using AAS.

Syntheses of serine complexes: To an aqueous solution of chloride salts of the metal ions of Co(II), Ni(II) and Cu(II), (1.133, 1.130, 0.812 g, 0.00476 M) respectively, ethanolic solution of L-serine (1 g, 0.00952 M) was added with constant stirring. The pH of the solution was adjusted by the addition of NaOH to 8-9 with total volume equal to 10 mL and the mixture refluxed for 2 h on a water bath. The coloured products obtained were collected by filtration and washed several times

with 80:20 ethanol-water mixtures and dried in an oven at 50 °C for 24 h. The reactions are summarized in eqn. 1.

$$MCl_2 + 2L + NaOH \longrightarrow [ML_2] \quad (1)$$

where, M = Ni(II), Co(II) and Cu(II), HL = L-serine.

Synthesis of mixed ligand complexes: To an aqueous solution of chloride salts of the metal ions Co(II), Ni(II) and Cu(II) (2.26, 1.26, 1.62 g, 0.00952 M) was added ethanolic solution of L-aspartic acid (1.266 g, 0.00952 M) followed by aqueous solution of L-serine (1.00 g, 0.00952 M) with constant stirring in 1:1:1 molar ratio. Equivalent amount of NaOH solution (0.381 g, 0.0095 M) was added; and the mixture refluxed for 2 h on a water bath. The coloured products were collected by filtration and washed several times with 80:20 ethanol-water mixtures and dried in an oven at 50 °C. The reactions are summarized in eqn. 2.

$$MCl_2 + L + L' + NaOH \longrightarrow Na[M(L'L)] \quad (2)$$

where, M = Ni(II), Co(II) and Cu(II), L = L-serine and L' = L-aspartic acid.

Antimicrobial activity using disc diffusion assay: Antimicrobial screening was carried out against two Gram-positive bacteria, *B. subtilis* and *S. aureus,* four Gram-negative bacteria: *E.coli*, *P.aeruginosa*, *K. pneumoniae* and *P. fluorescence* while antifungal screening was carried out against *C. pseudotropicalis*. The standard strains were from stocks of culture collections maintained at the pharmaceutics laboratory, Obafemi Awolowo University, Ile-Ife. The bacteria were maintained on nutrient agar slants and fungi on Sabouraud Dextrose Agar slants at 4 °C and subcultured monthly. Each test agent (10 mg) was dissolved in 1 mL sterile distilled water boiled gently in a Bunsen flame. Discs of Whatman No. 1 filter paper (6 mm) were soaked with 2 drops of the test agent using a sterile Pasteur pipette and allowed to dry at room temperature. Two colonies of 24 h plate culture of each organism were transferred aseptically into 10 mL sterile distilled water in a test tube and mixed thoroughly, using an electric shaker, for uniform distribution. A sterile cotton swab was then used to spread the resulting suspension uniformly on the surface of oven-dried Mueller Hinton Agar (Oxoid) and Sabouraud Dextrose Agar plates (Sterillin) for bacteria and fungi, respectively. These were incubated for 1 h at 37 °C and 25 °C for bacteria and fungi, respectively. Sterile forceps were used to aseptically place each of the disc on the agar plates and the plates were then refrigerated for 0.5 h at 4 °C following which, the inoculated plates were incubated at 37 °C for 24 h for bacteria strains and at 25 °C for 72 h for the fungal strain. Antimicrobial activity was evaluated by noting the zone of inhibition against the test organism [9].

RESULTS AND DISCUSSION

The physical properties of the synthesized complexes are given in Table-1.

Cobalt complexes: The transition metal complex ion Co(II) has a d^7 electronic configuration, with a spectroscopic ground state term ^4F in an octahedral field. It also has a ^4P term with the same spin multiplicity, which gives a T_1g spectroscopic state. The ^4F term is split into three sub energy levels namely $^4A_{2g}(F)$, $^4T_{2g}(F)$ and $^4T_{1g}(F)$. Transition can occur from $^4T_{1g}(F)$ sub-energy level (the lowest) to any of the others having the same spin multiplicity [10]. In the present case the *d-d* transition bands occurred at 520, 580 and 650 nm for metal complex of L-serine and 500, 530 and 600 nm for the mixed ligand complex of L-serine and L-aspartic acid and are assigned as $^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ transitions respectively (Table-2). The intra-ligand transition of L-serine and L-aspartic acid shifted to a longer wavelength (Bathochromic shift) on coordination from 203 and 210 nm to 217 and 215 nm for the complexes of L-serine and its mixed ligand, respectively.

TABLE-1
PHYSICAL DATA OF THE LIGANDS AND ITS COMPLEXES

Compound	Colour	Metal analysis found (Na)	Metal analysis found (M)
L'	White		
L	White		
$[Co(L)_2]$	Pink		19.17
$[Ni(L)_2]$	Green		18.63
$[Cu(L)_2]$	Blue		19.73
Na[Co(L'L)]	Lilac	7.58 (6.81)	17.78 (18.42)
Na[Ni(L'L)]	Green	7.37 (6.81)	18.25 (17.37)
Na[Cu(L'L)]	Turquoise blue	7.16 (6.71)	18.90 (19.57)

L = L-serine; L' = L-aspartic acid.

TABLE-2
ELECTRONIC SPECTRA BANDS FOR THE COMPLEXES

Compound	Intra ligand transitions (nm)	Ligand field transition (nm)	Assignment	Proposed structure
Ser	203		$n \rightarrow \pi^*$	
Asp	210		$n \rightarrow \pi^*$	
$[Co(L_2)]$	217	520 580 650	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)$ $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$	Octahedral
$[Ni(L_2)]$	237, 225	609 770 910	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$	Octahedral
$[Cu(L_2)]$	234	625	$^2E_g \rightarrow {}^2T_{2g}(F)$	Octahedral
Na[Co(L'L)]	215	500 530 600	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)$ $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$	Octahedral
Na[Ni(L'L)]	208	482 606	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$	Octahedral
Na[Cu(L'L)]	220	776 700	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ $^2E_g \rightarrow {}^2T_{2g}(F)$	Octahedral

Nickel complexes: The free ion ground state term for nickel(II) ion is ^3F. In an octahedral field three possible transitions are therefore expected, including the ^3P term. The *d-d* transition bands observed for the nickel(II) complex of L-serine were 609, 770 and 910 nm and for the mixed ligand complex of L-serine and L-aspartic acid were 482, 606 and 776 nm assignable to $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$, $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ with nickel ion in the octahedral ligand field respectively [10]. Shifts were observed in the intra-ligand transition bands to longer wavelength from 203 nm in the ligand, to 225-237 nm for L-serine complex. Shifts were also observed in the intra-ligand transitional band in the ligand at 210 nm to 208 nm on coordination with the mixed ligand complex.

Copper complexes: The electronic transition spectra for a Cu(II), d^9 complex is expected to show a single band. This is the case for a perfectly octahedral complex [10]. In the synthesized complexes, *d-d* transitions were observed at 625 nm for metal complex of L-serine and 700 nm for the mixed ligand complex of L-serine with L-aspartic acid assignable to $^2E_g \rightarrow {}^2T_{2g}(F)$ transition, which is more consistent with octahedral geometry. Shifts in the intraligand transition of L-serine and L-aspartic acid were observed at longer wavelength (Bathochromic shift) on coordination from 203 and 210 nm to 234 and 220 nm for the complex of L-serine and its mixed ligand complex respectively.

Infrared spectra: The infrared spectra assignment of the ligands and their metal complexes was achieved by comparing their vibrational frequencies with the values obtained in literature [8]. Relevant Infrared band frequencies are shown in Table-3.

L-Serine complexes: The O-H stretching vibrational frequency of L-serine at 3465 cm^{-1}shifted to shorter vibrational frequency (hypsochromic in the complexes) to 3416, 3462 and 3333 cm^{-1} for the Co(II), Ni(II) and Cu(II) metal complexes of L-serine. This indicates that the hydroxyl group of the L-serine participated in bond formation [11]. The N-H stretching vibration frequency was not observed in the free amino acid, serine, this may be ascribed to the zwitterionic nature of the ligand. On complexation, however N-H stretching vibration frequency was observed at 3238 and 3131 cm^{-1} for the cobalt and copper complexes respectively. For the [Ni(L_2)] complex the N-H_{str} vibration was not distinctive, suggestive of the anomalous behaviour of nickel [12]. The C=O stretching of the free amino acid L-serine occurred at 1605 cm^{-1} undergo a bathochromic shift to a longer wave number in cobalt and copper complexes to 1662-1641 cm^{-1} and to a shorter wave number a blue shift at1597 cm^{-1} for the nickel complex. The C-O stretching frequency observed at 1124 cm^{-1} in the spectrum of free ligand was found in the range 1158-1059 cm^{-1} of the complexes indicating the coordination of an oxygen atom of the carboxylate ion *via* oxygen the metal ion. Some new bands of weak intensity were observed in the regions around 703-669 cm^{-1} and 592-589 cm^{-1}, these may be ascribed to the M-O and M-N vibrations respectively [13]. It may be noted that these vibrational bands are absent in the infrared spectra of L-serine, which indicates that there is coordination between the metal and the lone pair of electron on the nitrogen and oxygen atoms of the ligands.

Mixed ligand complexes: The characteristic vibrational modes of the mixed ligand complexes are given in Table-3. An important feature of the spectra of the mixed ligand complexes is the absence of band at 3465-3422 cm^{-1} due to O-H stretching vibration of the free O-H group of serine. This was shifted to 3435, 3448 and 3429 cm^{-1} for Co(II), Ni(II) and Cu(II) complexes. Thus, suggesting that the O-H coordinated to the metal undeprotonated for L-serine. The N-H stretching vibration frequency was observed at 3250, 3277 and 3251 cm^{-1} for Co(II), Ni(II) and Cu(II) mixed ligand complexes of L-serine and L-aspartic acid respectively, suggesting coordination of the amino group through nitrogen with the metal ion. The C=O stretching vibrational frequency of the free amino acids occurred at 1605 and 1618 cm^{-1} was shifted to a higher wave number in the range 1627-1624 cm^{-1} in the spectra of the metal complexes for cobalt and nickel and to a lower wave number for the copper complex having 1616 cm^{-1} wave number, also C-O stretching vibration observed at 1124, 1069 cm^{-1} in the free amino acids undergo a bathochromic shift to a higher wave number on complexation in the range 1127, 1057 and 1186 cm^{-1} indicating the coordination of the carboxylate acid group *via* oxygen to the metal ion. Bands of weak intensity were observed in the regions around 625, 650 and 654 cm^{-1} and 577, 572 and 559 cm^{-1}, these bands may be ascribed to the M-O and M-N vibrations respectively [13] which further confirms the formation of the metal complexes.

Based on the foregoing it is suggested that both amino acids acted as tridentate ligands. L-Serine coordinating through the amino group, carboxylate oxygen and oxygen of hydroxyl group undeprotonated. On the other hand L-aspartic acid showed coordination through amino group and through the two carboxylate oxygen. Hence the structures shown in Figs. 1 and 2 are proposed for the complexes.

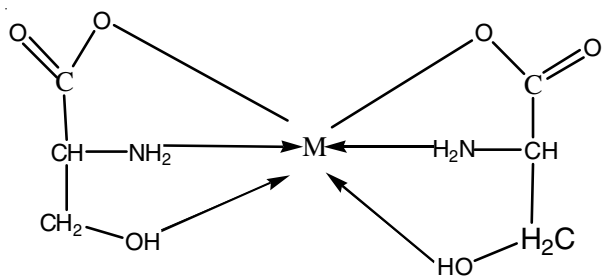


Fig. 1. Proposed structure of metal complexes of L-serine

Antimicrobial activity: The antimicrobial activities of the compounds are presented in Tables 4 and 5. Antimicrobial activities showed the synthesized compounds possess biological activity. The data revealed that the activities of the ligand are enhanced on complexation but less than the streptomycin. It has been observed that the ligands with nitrogen and oxygen donor systems inhibit enzymes production, since the enzymes

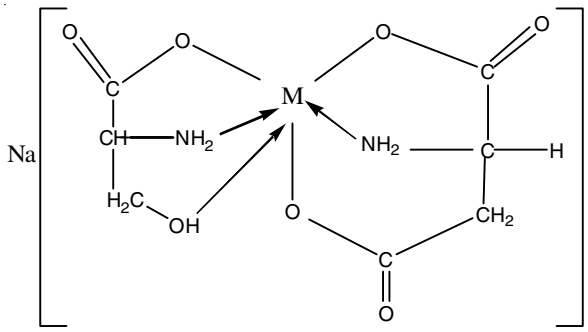
TABLE-3
INFRARED FREQUENCIES (cm^{-1}) FOR LIGANDS AND ITS COMPLEXES

Band	L	L'	[Co(L_2)]	[Ni(L_2)]	[Cu(L_2)]	Na[CoL'L]	Na[NiL'L]	Na[CuL'L]
O–$H_{Str.}$	3465(br)	–	3416	3462	3333	3435	3448	3429
N-$H_{Str.}$	–	3422(br)	3238(s)	–	3131	3250	3277	3251(m)
C=O_{Str}	1605	1618(s)	1641(s)	1597(s)	1662(s)	1627(s)	1624(s)	1616(m)
C-O_{Str}	1124 (s)	1069(s)	1059 (s)	1095(s)	1158(s)	1057(s)	1186(s)	1127(s)
M-O_{Str}	–	–	669(w)	648(m)	703(m)	625(m)	650(m)	654(m)
M-$N_{Str.}$	–	–	589(w)	592(m)	657(m)	577(m)	559(m)	572(m)

L = L-serine and L' = L-aspartic acid

TABLE-4
ANTIMICROBIAL ACTIVITY OF THE COMPLEXES AT 2 mg/mL (ZONE OF INHIBITION IN mm)

Micro organism	[Co(L_2)]	[Ni(L_2)]	[Cu(L_2)]	Na[CoL'L]	Na[NiL'L]	Na[CuL'L]	Streptomycin
B. subtilis			-	16	-	14	19
S. aureus	-	-	-	18	-	12	23
E. coli	-	-	15	-	14	15	23
P. aeruginosa	-	-	-	-	-	-	23
K. pneumoniae	-	-	-	-	-	-	19
P. fluorescence	-	-	20	-	-	10	23
S. flexinei	-	-	15	-	12	17	19
Streptomycin (1 mg/mL)							



where M = Co(II), Ni(II), Cu(II)

Fig. 2. Proposed structure of mixed L-serine and L-aspartic acid metal complexes

which require these groups for their activity appear to be more susceptible to deactivation by the metal ions upon chelation [14]. On chelation the polarity of the metal ions was reduced due to overlap of the ligand orbital and partial sharing of positive charges of metal ion with donor groups. The delocalization of π electrons over the whole chelate ring enhances the penetration of complexes into lipid membrane and blocking the metal bonding sites on enzymes of microorganism, hence increasing the biological activity [15].

TABLE-5
ANTIFUNGAL ACTIVITY OF THE COMPLEXES AT 2 mg/mL

	Zone of inhibition (mm)
Microorganisms	*C. pseudotropicalis*
Complex	–
Co(Ser)	–
Ni(Ser)	14
Cu(Ser)	–
Co(L'L)	–
Ni(L'L)	12
Cu(L'L)	18
Acriflavin (1 mg/mL)	21

Zone of inhibition is inclusive of the diameter of the cup.

The mixed ligand complexes exhibited better activity compared with the serine complexes with the exception of the copper(II) serine complex. The copper(II) serine complex for *E. coli* and *p. fluorescence* exhibited the best activity against Gram-negative bacteria. On the other hand, the mixed ligand complexes exhibited signi-ficant activity with the Gram-positive bacteria *B. subtilis* and *S. aureus*. Thus, indicating that the enhanced lipophilicity is due to the formation of the 6-membered ring of aspartic acid, may have aided their transfer to the active sites within the microbes.

Conclusion

The present study concluded that M-O and M-N bond and electronic spectra studies are indicative of coordination with octahedral geometry for the complexes, the mixed ligand complexes of L-serine and L-aspartic acid displayed antimicrobial activity towards the tested microbes, hence may be used to inhibit the growth of bacteria.

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