Antibacterial Studies of Leucine Complexes of Fe (III) and Cu (II)

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Abstract: ***Fe (III) and Cu (II) complexes of leucine were synthesised and characterized. The inhibition strengths of these complexes against some bacteria were evaluated. It was found that the $\lambda_{max}$of the complexes were at 670 nm and 650 nm for Cu-leu and Fe-leu respectively. There was an observed red shift between the λ_{max} of the metal salts and their complexes. This shift is a strong implication of coordination. The conductance of the complexes in water was 330.00µS and 17.60 µS for Cu-leu and Fe-leu respectively. The IR frequencies of the leucine due to $^+NH_3$ group found at 1533.46 cm^{-1} and 2616.53 cm^{-1}disappeared in the complexes, indicating coordination through $^+NH_3$. Also the peaks for ν_s (COO$^-$) in the leucine was 1389.76cm^{-1}. This band was decreased to 1379.15 cm^{-1} and 1385.90 cm^{-1} for Cu-leu and Fe-leu respectively. These changes also portend coordination of the leucine to these metal ions via carboxylate. Again, IR bands for Cu-leu and Fe-leu at 3241.48 cm^{-1}and 3372.66 cm^{-1} are firm evidence of the presence of coordinated water molecules in the complexes. The Fe and Cu ions are thus coordinated to the ligand via carboxylate (COO$^-$) and H_3N^+groups in a bidentate fashion. Octahedral and square pyramidal geometry were proposed for the Fe-leu and Cu-leucomplexes respectively. The results of the antimicrobial screening by agar diffusion with these amino acids complexes showed increased antibacterial activities when compared to their salts.***

Keywords: Antibacterial, Leucine, Complexes, Coordination, Conductance

1. Introduction

The chemistry of transition metal complexes is well known. However, the evaluation of their antimicrobial activities has continued to attract more and more attention. This is because bacteria can cause foodborne disease[1] and also affect our lives; therefore, there has been constant effort to derive new antimicrobial agents [2][3].Coordination complexes of transition metals have been widely studied for their antimicrobial activities[3][4].Mixed ligand complexes of Zn(II) and Co(II) with cysteine, histidine, cysteinemethylester, and histidinemethylester were synthesized[5]. Histidine and histidinemethylester acted as bidentate ligands involving amino and imidazole nitrogen in the complexes[5]. Alsothe cysteine and cysteinemethylester acted as bidentate ligands coordinating via thiol sulphur and amino nitrogen. Tetrahedral geometry was proposed for Zn(II) and Co(II) complexes[5]. Corbi *et al.*,[6]prepared solid Ni(C_5 H_{10} NO_3 $S)_2$ .2H_2O complex and demonstrated that its electronic absorption spectrum showed an octahedral geometry. The infrared spectroscopy analysis showed that the metal atom coordinated to the ligand through (COO$^-$) and (S = O) groups, while the thermal analysis revealed the presence of coordinated water molecules [6]. The complex also showed high solubility in water[6]. The antibacterial properties of copper and cobalt amino acids complexes on agar plates were investigated [7]. The complexes were mainly active against gram-positive organisms, species like *Micrococcus luteus* were the most susceptible strain tested. They acted moderately against *Bacillus cereus*[7]. The microorganisms *Escherichia coli*, which are known to be multi-resistant to drugs[7], were also resistant to the amino acids complexes[7]. *Escherichia coli*were susceptible only to the $CoCl_2$ and copper complex with phenylalanine. The complexes with leucine and histidine were more active [7]. Weak activity was shown in the case of complexes with methionine andphenylalanine[7]. In addition, metallic copper surfaces have been found to have antibacterial activity against *S. enterica* and *C. jejuni*[1].Therefore, the aim of the present study is tosynthesize Cu (II) and Fe (III) leucine complexes and evaluate their bacterial properties on some strains.

2. Materials and Methods

2.1 Materials

Leucine, FeSO4. 7H_2O (Kermel), $CuSO_4$ 5H_2O (M & B), 6305 UV-visible spectrophotometer, Bench pH/Conductivity meter (PC 510), FTIR 8400S Spectrophotometer and Distilled water were used for the preparation and characterisation of the complexes.

2.2 Preparations of the Leucine Complexes of Fe (III) and Cu (II)

The complexes were prepared following a previously reported procedure. 2 mmols of leucine was dissolved in 20 mL distilled water and for deprotonation of the amino acids 0.33 mL 30% NaOH was added and stirred for several minutes [7][8]. Then 1 mmol of the metal salts was dissolved in 2 mL of distilled water and was added to the deprotonated amino acid and stirred at room temperature for 2 hrs. There was precipitation of the complexes. The precipitate was filtered off, washed with water several times, and dried in air. The colour was dark - brown for Fe (III) complex and blue for copper complex.

2.3 Antibacterial Studies

Agar diffusion method was used to assess the bacterial activities of the salts and leucine complexes of these transition metals. The bacteria were; *Mycobacterium tuberculosis, Bacterial Vaginosis, Pseudomonasaeruginosa, Salmonella Typhi, Escherichia coli, Campylobacter spp*.

3. Results and Discussion

The results of Physico-bacterial characterization of the complexes are presented in and Figures 1, 2 and Table 1 as follows:

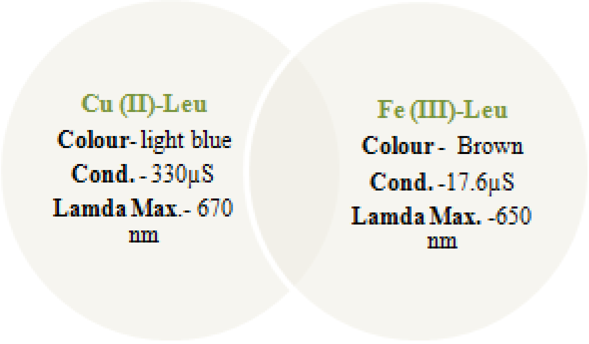

Figure 1: Physico-Chemical Characterization of Cu (II) and Fe (III) Leucine Complex

The wavelength at which the complexes showed maximum absorbance was observed at 670nm and 650nm for Cu-leu and Fe-leu respectively. There was an observed red shift between the λ_{max} of the metal salts and their complexes[6]. That likely implies the formation of complexes between the amino acids and the metal ions. The conductance of the complexes in water was 330. 00 µS and 17.60 µS for Cu-leu and Fe-leu respectively; these are regarded to be low as compare to previous work [6]. According toCorbi *et al.*, [6], the Ni(II) complex with methionine sulfoxide showed extremely high solubility in water, differing from the Mn (II), Co(II), Cu(II) and Ni(II) complexes with methionine $[M(Met)]_n$, which are insoluble[6].

Table 1: Infrared absorption frequencies (cm^{-1}) of Leucine, Fe-leucine and Cu-leucinecomplexes

Leu	Cu(II)-leu	Fe(III) - leu	Assignts.	Ref.
-	3241.48	3372.66	v (O-H)	(9)
1600.01	1619.29	1605.79	v_{as} (COO$^-$)	(10)(9)
1533.46	-	-	δ_s(NH$_3^+$)	(11)
2616.53	-	-	v_s (NH$_3^+$)	(11)
1389.76	1379.15	1385.90	v_s (COO$^-$)	(10)
-	386.74-572.88	405.06-659.68	v(M-N, M-O)	(10)

The IR frequencies of $^+NH_3$ in the ligands (leucine) observed at 1533.46 cm^{-1}, 2616.53 cm^{-1} were found to disappear in the spectra of the complexes (Table 1). This is an indication of the coordination of the leucine via N-atom[11]. Also the IR peak for symmetrical stretching of the ionized carbonyl v_s (COO$^-$)in the leucine was 1389.76cm^{-1}. This band was shifted downward to 1379.15 cm^{-1} and 1385.90 cm^{-1} for Cu-leu and Fe-leurespectively[10]. In addition, the asymmetric carbonyl stretchv_{as} (COO$^-$) in the leucine was at1600.01cm^{-1}, and this was increased to 1619.29cm^{-1}for Cu-leu and 1605.79cm^{-1}for the Fe-leu. This furtherindicates the coordination of leucine by the metal ions[9][10]via the carboxylate group of atoms. Furthermore, IR bands for Cu-leu and Fe-leu at 3241.48 cm^{-1}and 3372.66 cm^{-1} are suggestive of the presence of coordinated water molecules in the complexes[10]. Thus,the ligand (leucine) is bidentately coordinated to the metals ions via carboxylate carbonyl (COO$^-$) and $^+NH_3$, in an octahedral and square pyramidal arrangements for Fe-leu and Cu-leu respectively [6][12]. These IR frequencies of 386.74-572.88 cm^{-1}found inCu-leu and 405.06-659.68 cm^{-1}inFe-leu may be further indication of the coordination due v(M-N, M-O) vibrationsin the complexes[10][13].

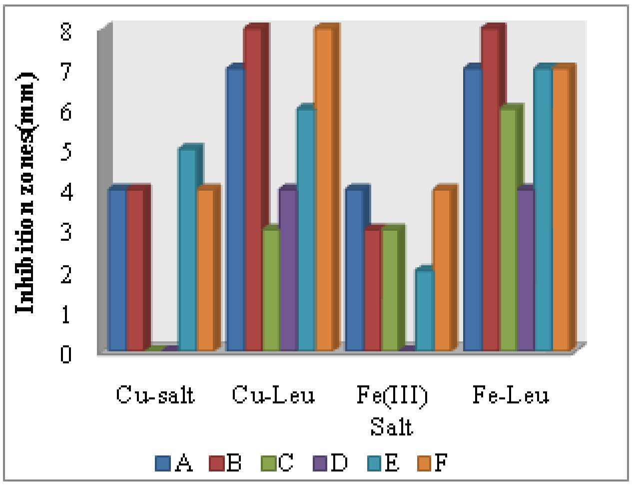

Figure 2: Levels (mm) Inhibition zones

A = *Mycobacterium tuberculosis;* B= *Bacteria vaginosis;* C= *Pseudomas aeruginosa;* D= *Salmonella typhi;* E = *Escherichia Coli;* F = *Campylobacter spp*

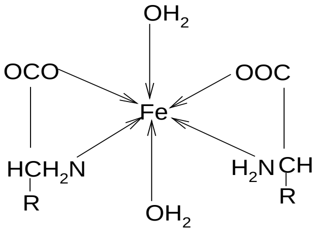

Figure 3: Proposed structures of the *Fe-leu complex*

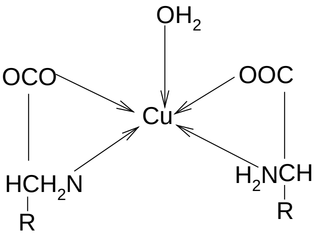

Figure 4: Proposed structures of theCu-leu complex

The results of the antimicrobial screening by agar diffusion are shown in Figure 2. These amino acids complexes showed increased antibacterial activities when compared to the original metal salts. The details of the results are given below: for *Mycobacterium tuberculosisi*, the activities of these materials were; Fe(III)-leu = Cu-leu> Cu(II) salt = Fe (III) Salt; *Bacteria vaginosis*, Fe(III)-leu = Cu-leu> Cu(II) salt > Fe (III) Salt; *Pseudomas aeruginos*, Cu(II) salt <Cu-leu = Fe (III) Salt < Fe(III)-Leu; *Salmonella typhi*, Cu-leu = Fe(III)-leu but no inhibition was observed with their salts;

Escherichia Coli, Fe(III)-leu >Cu-leu> Cu(II) salt > Fe (III) Salt. The studies show that, the activities of these agents against *Campylobacter* was as follows, Cu-leu> Fe(III)-leu > Cu(II) salt = Fe (III) Salt. Copper and ironleucine complexes in our hands were observed to have relatively same antibacterial activities but higher than their respective salts.This studies emphasis the use of Cu-leu complex as newly alternative antibacterial therapeutic agents. It has been reported that the antibacterial activity of a complex is influenced by its stability. The lower stability of the amino acid complex, the greater is the antibacterial activity. This is probably because they have more free ions in the solution, which can enhance the cooperative interaction between the metal ions and the ligands[8]. Another possibility linked to the biological activities of transition complex is that, the positive charge of the metal is partially shared with the donor atoms present in the ligands and there is π-electron delocalization over the whole chelate ring. This in turn increases the lipophilic character of the metal chelate and favours its permeation through the lipid layers of the bacterial membranes[1][7][10]

4. Conclusion

In this work leucine complexes of Fe (III) and Cu were synthesised. Leucine was observed to coordinate with the metal atoms via $^{+}NH_3$ and COO^{-} groups. Octahedral (Fe-leu) and square pyramidal (Cu-leu) structures were proposed for the complexes prepared. The results of the antimicrobial screening by agar diffusion showed increased antibacterial activities using the Cu-leu and Fe-leu as compare to their salts.

5. Recommendations

This revealed that Leucine can be used as antidote or chelating agent for medical treatment of metals overload or poisoning**,** whereas these complexes can find used as antibacterial agents. These complexes could be characterized using different techniques, such as elemental analysis, thermogravimetric analysis, magnetic measurements, NMR and X-ray crystallography in subsequent researches for detail structural elucidation. This may enhance their applications and uses.

6. Acknowledgements

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