IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 9, Issue 11 Ver. III (November. 2016), PP 01-06
www.iosrjournals.org

Facile Eco-friendly Synthesis, Spectral and Antimicrobial Activities of Copper - Amino Acid Complexes

K.P. Srivastava* & Anuradha Singh
Department of Chemistry, N.L.S.College, Jaitpur-Daudpur (Saran) Jai Prakash University, Chapra-841301, Bihar, INDIA

Abstract: *A rapid, efficient, clean and environmentally benign exclusive synthesis of copper (II) complexes with non-essential biologically active amino acids [L-Asparagine (Asn or N), L-Glutamine (Gln or Q) and L-aspartic acid or aspartate (Asp or D)] efficiently in a water -ethanol suspension medium using alkali catalyst with excellent yields has been described here. All the amino acids were bidentate (N & O donors) ligands that were used for complexation with Cu^{2+} ion. All the eco-friendly synthesized complexes were characterized by analytical and spectral methods. The ν(C-O) and ν(N-H) vibrations were shifted toward higher frequencies for complexes comparable with ligands. Visible electronic and NMR spectra at room temperature are typical for monomeric species with square planner symmetry around the metal ion. The synthesized complexes were evaluated for their in vitro antibacterial activity against five bacteria stains and one fungi by the agar-well diffusion method. The investigated amino acids were found to exhibit low to moderate activity but all the complexes exhibited varied vigorous activity against different bacteria. The amino acids which were less active before complexation became more active upon coordination with mentioned bivalent transition metal ion.*

Keywords: *Copper, α-amino acids, antibacterial activity, square planner geometry*

I. Introduction

The development of more potent metal-based drugs have been investigated over the last three decades and it has been discovered that inorganic compounds have enormous impact in medicine [1]. Some metal complexes have been found to have antimicrobial and antiviral properties and could be effective against diseases [2]. This had led to numerous investigations on metal-drug interactions, and more studies on metal complexes with the aim of discovering more effective chemotherapeutic agents to fight diseases. Also, it is known that some drugs act via chelation or by inhibitory metalloenzymes but for most drugs that act as potential ligands, a lot of studies are being carried out to know how metal binding influences their activities [3]. As therapeutics, inorganic compounds are used as chelating agents and as chemotherapeutics: anticancer agents, metal-mediated antibiotics, antibacterial, antiviral, anti-parasitic, treatment of rheumatoid arthritis and radio-sensitizers [4-5].

The study of amino acid-metal complexes is increasingly becoming important particularly in drug design and nutrition. This has led to lots of study on amino acid – metal complexes, with the hope of improving and enriching the quality of existing vitamins, thereby serving as a better substitute as chemotherapeutic agents. In the search for novel metal complexes, that combine high activity with low toxicity, the study of metal complexes has continued to attract attention of some coordination chemists [6-7].This has also provided useful outlets for basic research in coordination chemistry.

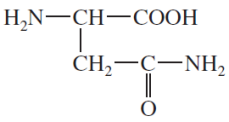
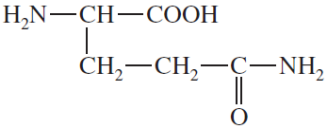
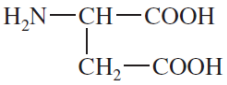
The standard amino acids are 20 common α-amino acids that are the chemical units of nearly all proteins, which construct the structure for all living organism and essential for various biochemical processes that support the maintaining of life in the individuals [8-9]. All the standard amino acids are L-amino acids. They are good chelating agents [10-11] and can coordinate to transition metals through their amino and or carboxylic groups. During the last few decades the complexation of transition metal ions with amino acids have been studied [12-14]. The amino acid-metallic ion interactions are found to be responsible for enzymatic activity and stability of protein structures. The metal-amino acid complexation is important field of study as they can be used as representative model systems to understand the metal-protein interaction in biological systems and metabolic enzymatic activities [15].

Copper(II) is a biologically active, essential ion; its chelating ability and positive redox potential allow participation in biological transport reactions. Also, Cu(II) forms the active centers of more than a dozen metalloproteins . Further, copper complexes possess a wide range of biological activity and are among the most potent antiviral, antitumor and antiinflammatory agents [16-17]. These biological activities have provided an impetus to the study Cu(II) complexes of amino acids in general, and have prompted us to update the structural-activity correlations. As a result of resistance to the drugs currently in use and the emergence of new diseases, there is a continuous need for the synthesis and identification of new compounds as potential antimicrobial agents. Therefore we considered it necessary to study the effects of the possible varying

structures of Cu(II)-complexes of some amino acids and their antimicrobial activity, as this would yield information useful for designing antimicrobial agents.

The present work is in continuation of our research on coordination complexes of drug derivatives with transition metals and evaluating their biological activity [18-19]. The aim of the present study was to evaluate the antimicrobial properties of eco-friendly synthesized a range of copper (II) complexes with different amino acids comparing with free ligands ligands(L-*Asn*, L-*Gln* and L-*Asp*)[**Table-1**].

Table-1: Amino Acids used as Ligands

Name	**L-Asparagine**	**L-Glutamine**	**L-Aspartic Acid**
Structure			
Symbol	N	Q	D
Abbreviation	*Asn*	*Gln*	*Asp*
Isoelectric Point	5.4	5.7	2.8

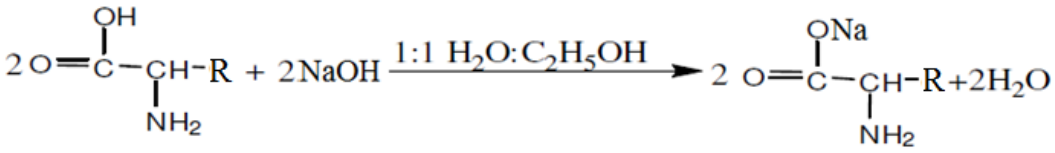
II. Experimental

Materials and Methods

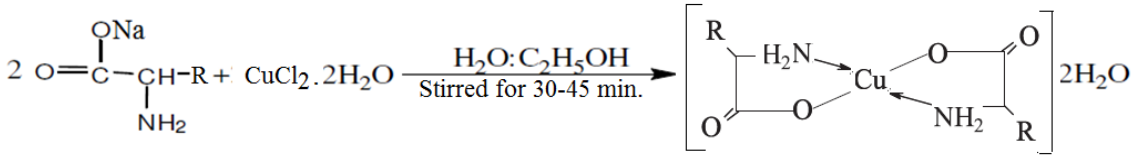
The salt $CuCl_2.2H_2O$ used in this study was of AR grade and purchased from E. Merck. The amino acids *L-Asparagine (Asn or* N), *L-Glutamine (Gln or* Q*) and L-aspartic acid or aspartate (Asp or* D*)* were purchased from Loba Chemie which were used as received without further purification. The metal salt and amino acid solutions were prepared by direct dissolution in doubly distilled water.

Synthesis of Cu-Complexes

A solution of $CuCl_2.2H_2O$ (1 mmole) in 1:1 mixture of ethanol and water (10 mL) was added to a solution of the ligand (4 mmol) in 20 ml H_2O/ethanol(50%) mixture containing 0.33 mL 30% NaOH (for deprotonation of the amino acids) [20] using stoichiometric amount (1:2) [(metal: $2(Na^+$ L)] molar ratio. The reaction mixture was stirred for several minutes (30-45 minutes) at room temperature. After 1-2 hours a coloured crystalline solid was obtained which was filtered and washed with water-ethanol then triethyl ether .The solids were recrystallized from (H_2O:DMSO) (30:70) volumes' mixture and dried in vacuum over anhydrous $CaCl_2$ (Scheme-2) at 60^OC. The yields range from 85 to 90 %. The compounds were found to be soluble in DMSO and hot water.



Scheme-1: Deprotonation of used amino acid ligands



where R = $CH_2.CONH_2$ for N (*Asn*), $CH_2.CH_2.CONH_2$ for Q (*Gln*) and R = $CH_2.COOH$ for D (*Asp*)

Scheme-2: Schematic representation of synthesis of the Cu(II) Complexes

Instrumentation

Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. All compounds were analysed satisfactorily for C, H and N using Carl-Ebra 1106 elemental analyser in micro-analytical laboratory. Metal content was estimated complexometrically by standard procedure [20-22]. Molar conductance measurements were conducted using 10^{-3}M solution of the complexes in water on Elico-CM 82 Conductivity Bridge at room temperature. Gouy balance at room temperature using mercuric tetrathiocyanato

cobaltate(II) as the calibrant. Diamagnetic corrections were applied in compliance with Pascal's constant [23]. The pH of the solutions was measured by using Elico Li-120 pH meter.

The electronic absorption spectra of all the complexes in DMSO solution (10^{-3} M) in the ultraviolet and visible region were recorded on Shimadzu UV/VIS-160 spectrophotometer. The FT-IR spectra were recorded in KBr discs on a Perkin Elmer Spectrometer-577 in wave number region 4000-200 cm^{-1}.

Antibacterial screening by Agar cup method

The strains used were *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Bacillus subtilis*, and one Methicillin resistant *S. aureus* clinical isolate for bacteria and *C. albicans* for fungi. In the agar cup method, a single compound can be tested against number of organisms or a given organism against different concentrations of the same compound. The method was found suitable for semisolid or liquid samples and was used in the present work. In the agar cup method, a plate of sterile nutrient agar with the desired test strain was poured to a height of about 5 mm, allowed to solidify and a single cup of about 8 mm diameter was cut from the centre of the plate with a sterile cork borer. Thereafter, the cup was filled with the sample solution of known concentration and the plate was incubated at 37^{O}C for 24 h. The extent of inhibition of growth from the edge of the cup was considered as a measure of the activity of the given compound. By using several plates simultaneously, the activities of several samples were quantitatively studied. All the tests were performed in triplicate.

III. Results And Discussion

Characterization of metal complexes

The synthesis of mixed ligand Cu(II) complexes may be represented in scheme-2. All the complexes are coloured, non-hygroscopic and thermally stable solids (Table-1), indicating a strong metal-ligand bond. The complexes are insoluble in common organic solvents such as ethyl alcohol, acetone, etc., but are fairly soluble in DMSO and in hot water. The elemental analysis data (Table-2) of metal complexes are consistent with their general formulation as 1:2, monomeric complexes of the type $[Cu(L)_2].2H_2O$. The molar conductance values of the complexes in DMSO at 10^{-3} M concentration are very low indicating their non-electrolytic nature [24]. The melting points or decomposition temperatures for the complexes are shown in table-2. Most of the complexes decomposed before melting.

Magnetic studies

The magnetic moments of the metal complexes were calculated from the measured magnetic susceptibilities after employing diamagnetic corrections and revealed their paramagnetic nature. The observed values for effective magnetic moment (μ_{eff}) in BM, reported in table-3, suggest the square planar geometry for copper complexes [25]. The magnetic moments of the investigated complexes were slightly greater than spin only values due to Jahn-Teller's effect and spin-orbit coupling respectively and support the conclusions.

Table-2: Empirical formula, molecular weight, colour, decomposition temperature and pH of the investigated copper complexes

Complex	**Empirical Molecular**	**formula weight**	**Colour**	**Decomposition temperature (^{O}C)**	**pH**	**% Yield**
$[Cu(Asn)_2].2H_2O$	$[Cu(C_4H_7N_2O_3)_2]$	325.5	Blue	217	**7**	85
$[Cu(Gln)_2].2H_2O$	$[Cu(C_5H_9N_2O_3)_2]$	353.5	Blue	198	6	88
$[Cu(Asp)_2].2H_2O$	$[Cu(C_4H_6NO_4)_2]$	327.5	Blue	205	**6**	90

Table-3: Elemental analysis data, molar conductance and magnetic moments of investigated copper complexes

Complex	**Elemental analysis Found (Calcd.)**				**Molar conductance (ohm^{-1} cm^2 mol^{-1})**	**μ_{eff} (in BM)**
	%M	**%C**	**%H**	**%N**		
$[Cu(Asn)_2].2H_2O$	19.62 (19.50)	29.54 (29.49)	4.40 (4.30)	17.32 (17.20)	0.12	1.86
$[Cu(Gln)_2].2H_2O$	18.02 (17.96)	34.04 (33.94)	5.12 (5.09)	15.95 (15.84)	0.18	1.85
$[Cu(Asp)_2].2H_2O$	19.42 (19.38)	29.36 (29.31)	3.72 (3.66)	8.60 (8.55)	0.28	1.89

Electronic Spectral Study

The electronic spectra of the ligands showed three absorption bands at 187-196, 210-216, and 223-232 nm assigned as the $n \rightarrow \sigma^*$, $n \rightarrow \pi^*$, and $\pi \rightarrow \pi^*$ transitions of the major chromophores, NH_2 and COO−, present in the ligand molecules. On coordination, however, shifts were observed in these bands in addition to *d-d* transitions bands (Table-4). These in conjunction with the magnetic moment of the complexes were used to propose probable geometry of the complexes obtained.

The electronic spectra of the investigated complexes in DMSO were recorded in the UV-visible region. The spectra for the investigated copper(II) complexes displayed two bands at 615-628 and 654-667 nm, , assigned to $^2B_{1g} \rightarrow {}^2E_g$ and $^2E_g \rightarrow {}^2A_{1g}$ *d-d* transitions. This was the indicative that the investigated complexes were the mononuclear complexes with 4-coordinate square planar geometry [26]. This proposed geometry was corroborated by their magnetic moment value of 1.85 -1.89 BM. The important electronic spectral bands along with their assignments of the isolated ligands and the complexes under investigation are listed in table-3.

Table-4: Electronic spectral bands and proposed geometry of the Cu(II) complexes

Compound	**nm (□, cm², mol⁻¹)**		**□eff (in BM)**	**Proposed geometry of complexes**
	Ligand bands	**d-d bands**		
Asn	187, 200, 210	--	--	--
Gln	192, 200, 215	--	--	--
Asp	196, 212, 232	--	--	--
$[Cu(Asn)_2].2H_2O$	--	615, 662	1.86	D_{4h}
$[Cu(Gln)_2].2H_2O$	--	623, 656	1.85	D_{4h}
$[Cu(Asp)_2].2H_2O$	--	628, 667	1.89	D_{4h}

Infra-red spectral Study

The FTIR spectra of the metal complexes were recorded in KBr discs over the range 4000-400 cm-1. These spectra were complex due to the presence of numerous bands with varying intensities, making the interpretation task quite difficult. However, an attempt has been made to assign some of the important bands on the basis of reported infrared spectra of several N- and O-donor ligands, and their metal complexes (Table-5).

Table-5: Relevant IR bands for the compounds

Compound	**□s(NH2)**	**□s(COO⁻)**	**□asy(COO⁻)**	**□(M-N)**	**□(M-O)**
Asn	3410m	1648s	1585s	--	--
Gln	3390s	1645s	1594s	--	--
Asp	3380w	1650s	1583s	--	--
$[Cu(Asn)_2].2H_2O$	3420w	1590s	1495m	535	624
$[Cu(Gln)_2].2H_2O$	3424m	1585w	1505w	548	645
$[Cu(Asp)_2].2H_2O$	3433m	1541w	1509w	552	656

w: weak; m: medium; s: strong

The infrared spectrum of the free ligand exhibited a broad band at 3380-3410 cm^{-1} which was assigned to the NH_2 stretching frequency. Intense bands at 1645-1650 and 1583-1594 cm^{-1} were observed and are attributed to COO^-_{asy} and COO^-_{sy} stretching frequencies, respectively [27-28].

For the investigated complexes the COO−asymmetric stretching frequencies were shifted to lower frequencies compared with that of the ligand. Bands in the region of 624– 656 cm^{-1} indicate the formation of M–O bond and further support the coordination of the ligand to the central metal ion via the oxygen atom of the carboxylate group [29]. Hypsochromic shifts were observed for the $–NH_2$ frequencies on coordination, for the investigated complexes. This indicates bond elongation on coordination. It therefore suggests probable square planar geometry for the complexes. New bands in the spectra of the complexes at 535–552 cm^{-1} were assigned to (M–N) stretching frequency. The participation of the lone pairs of electrons on the N of the amino group in the ligand in coordination is supported by these band frequencies [30].

^{1}H-NMR Spectral Study

The NMR spectroscopy is very important tool of investigation of structure of an unknown compound. This was studied using TMS as standard reference. The ^{1}H-NMR spectra in the ligands and of the investigated complexes were of the type (I) and (II).

In all the ligands, signal of -COOH appeared at -0.95τ (off the scale) but in metal complexes these signals do not appear, probably due to replacement of H+ by metal ion. The signal due to protons of NH2 appeared perturbed (decreased) probably due to coordination by N of NH2 to metal ion. The NMR spectral stud clearly shows the formation of complexes by the ligands. The NMR spectral results are presented in table-6

Table-6: Relevant 1H-NMR signals for the compounds

Type of protons (In Ligands)	Nature of signal	Chemical shifts	
		δ	τ
-COOH	singlet	10.95	-0.95
-CONH2	Broad singlet	8	2
-NH2	Broad singlet	6	4
=CH	quartet	4.2	5.8
-CH2	pentate	2.1	7.9
(In Complexes)			
-COOH	--	--	--
-CONH2	Broad singlet	8	2
-NH2	Broad	6.2	3.8
=CH	quartet	4.2	5.8
-CH2	pentate	2.1	7.9

Proposed Structures

On the basis of the above observations, it is tentatively suggested that transition metal(II) investigated complexes show a square planar geometry [**Figure-1**] in which the ligands act as mono-negative bidentate [N & O donor] ligands.

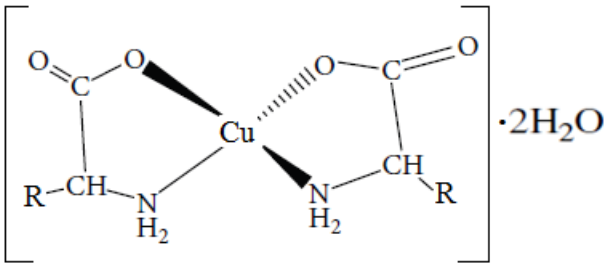


Figure-1: Tentative square planar structure for investigated complexes

Anti-bacterial Activity

The difference in anti-bacterial activities of the investigated complexes and ligand with the standard were studied and the results are presented in **table-7.** The synthesized complexes were found to be more active compared with their respective free ligands against the same microorganisms and under the identical experimental conditions and in some cases better activity compared to the standard. The increase in biological activity of the metal chelates may be due to the effect of the metal ion on the normal cell process. A possible mode of toxicity increase may be considered in the light of Tweedy's chelation theory [31-32]. Chelation considerably reduces the polarity of the metal ion because of partial sharing of its positive charge with the donor group and possible p-electron delocalization within the whole chelate ring system that is formed during coordination. Such chelation could enhance the lipophilic character of the central metal atom and hence increase the hydrophobic character and lipo-solubility of the complex favouring its permeation through the lipid layers of the cell membrane [33-34]. This enhances the rate of uptake or entrance and thus the antimicrobial activity of the testing compounds. Accordingly, the antimicrobial activity of the complexes present in this investigation can be referred to the increase of their lipophilic character which in turn deactivates enzymes responsible for respiration processes and probably other cellular enzymes, which play a vital role in various metabolic pathways of the tested micro-organisms.

Table-7: Antimicrobial activities of Ligands and its Cu(II)-complexes

Compound	***E. coli***	***P. aeruginosa***	***P. vulgaris***	***S. aureus***	***B. subtilis***	***C. albicans***
Asn	8.09	6.04	6.07	7.10	7.05	8.05
Gln	8.03	7.02	6.07	6.09	7.01	8.03
Asp	6.02	6.07	6.0 0	6.01	6.01	8.01
$[Cu(Asn)_2].2H_2O$	7.15	5.50	5.09	6.08	6.90	7.06
$[Cu(Gln)_2].2H_2O$	7.08	6.00	6.01	6.05	5.09	7.90
$[Cu(Asp)_2].2H_2O$	6.00	6.03	6.02	6.07	7.00	6.03
Acriflavine	20.04	6.00	15.06	20.02	20.02	19.01

IV. Conclusion

We report here the eco-friendly syntheses of coordination compounds of Cu(II) ion with some amino acids in basic media and their characterization and antimicrobial activities.

The copper complexes of amino acids have been prepared in an environmentally benign protocol and characterized by elemental analysis, conductivity measurements, magnetic moment, spectral analysis. Elemental analysis of the complexes reveal 1:2 metal to ligand stoichiometry and non-electrolytic nature. Physico-chemical studies reveal that the ligands undergoes complexation and gets coordinated through nitrogen of the α-amine and β- carboxylate oxygen. The ligand behaves as a mono-negative bidentate donor (N & O) yielding four coordinated transition metal complexes with square planer geometry around the central metal ion. The synthesized complexes were found to be more active compared with their respective free ligands against the same microorganisms and under the identical experimental conditions and in some cases better activity compared to the standard.

Present methodology offers very attractive features such as simple experimental procedure, higher yields and economic viability, when compared with other method as well as with other catalysts, and will have wide scope in organic/inorganic syntheses.

References

[1]. N. B. Behren, G. M. Diaz, D. M. L. Goodgame, **1996**, *Inorg. Chim, Acta*.,125, 21–26.
[2]. J. A. Obaleye, C. L. Orjiekwe, D.A. Edward, **2000**, *Synth. React. Inorg. Met-Org. Chem*., 30(4), 735 – 744.
[3]. C. Orvig, M. J. Abrams, Eds. **1999**, *Medicinal Inorganic Chemistry Chem. Rev*., 99(9), 2201 – 2842.
[4]. Z. Guo, P. J. Saddler, **1999**, *Angew. Chem. Int. Ed., Engl*., 38 (II), 1512 – 1531.
[5]. M. J. Abram, B. A. Murrer, **1993**, *Science*, 261, 725 – 730.
[6]. N. Wasi, H. B. Singh, **1987**, *Inorg. Chim. Acta*, 135, 133-137.
[7]. P. Kopf- Maier, **1994**, *Eur. J. Clin, Pharm*., 47 (1), 1 – 16.
[8]. E. Canpolat, M. Kaya, **2004**, *Turk. J. Chem*., 28, 235-242.
[9]. N. Farrell, **2003**, Metal complexes as drugs and chemotherapeutic agents; *Comprehensive Coordination Chemistry*, 9, 809-840.
[10]. Z.H. Chohan, M. Arif, M.A. Akhtar, C.T. Supuran, **2006**, *Bioinorganic Chemistry and Applications*, 1-11.
[11]. K. Nomiya, S. Takahashi, R. Noguchi, S. Nemoto, T. Takayama, M. Oda, **2000**; *Inorganic Chemistry*, 39, 3301-3311.
[12]. A. Legler, A. Kazachenko, V. Kazbanov, O. Per' yanova, **2001**; *Pharmaceutical Chemistry*, 35, 35-36.
[13]. T. Aiyelabola, I. Ojo, O. Akinkunmi, **2012**; *International Journal of Chemistry*, 4, 49-59.
[14]. A. Stanila, A. Marcu, D. Rusu, M. Rusu, L. David, **2007**; *Journal of Molecular Structure*, 834-836, 364-368.
[15]. J. Balla, P. V. Bernhardt, P. Buglyo, P. Comba, T. W. Hambley, R. Schmidlin, et al., **1993,** *J. Chem.Soc., Dalton Trans.*, 1143-1149.
[16]. H. Sigel (Ed.), *Metal Ions in Biological Systems*, Vol. 13, Dekker, New York, **1981**.
[17]. K.D. Karlin and J. Zubieta, (Eds.), *Biological and Inorganic Copper Chemistry*, Adenine Press, New York, **1986**.
[18]. K.P.Srivastava, Anuradha Singh, Suresh Kumar Singh, **2014**, *IOSR Journal of Applied Chemistry;* **2014**, 7, 4(1), 16-23.
[19]. K.P.Srivastava, Anuradha Singh, Suresh Kumar Singh, **2014,** *Int. J. Adv. Res. Chem. Sci.*, 1 (2), 11-20.
[20]. A. Stanila, A. Marcu, D. Rusu, M. Rusu, L. David, **2007**, *J Mol Struct*., 834:364-368.
[21]. A.I. Vogel, *Textbook of Quantitative Inorganic Analysis*, 5th edn., Longmans Green and Co. UK Ltd., London, **1989**.
[22]. A.I. Vogel, *Quantitative Inorganic Analysis*, 4th edn. ELBS, London, **1965**.
[23]. R.L. Dutta; A. Syamal, *Elements of Magneto Chemistry*, 2nd edn. Affiliated East West Press, New Delhi, **1993**.
[24]. W.J. Geary, **1971**, *Coord. Chem. Rev*. 7, 81.
[25]. A.D. Lieher *J. Phys. Chem.* **1967**, 67, 1314.
[26]. C. J. Ballhausen, *An Introduction to Ligand Field Theory*, McGraw-Hill, NY, USA, 1962.
[27]. D. Pavia, G. Lampman, G. Kriz, *Introduction to Spectroscopy, A Guide for Students of Organic Chemistry*, pp. 22–368, Brooks and Cole, New York, NY, USA, 3rd edition, **2001**.
[28]. K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, K. Nakamoto, Ed., pp. 66–74, Wiley Interscience, New York, NY, USA, **2009**.
[29]. W. Kemp, *Organic Spectroscopy*, pp.22–38, Macmillan, Hong Kong, **1991**.
[30]. L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, Chapman & Hall, London, UK, **1975**.
[31]. B. G. Tweedy, *Phytopathology*, **1964**, 55, 910.
[32]. T. O. Aiyelabola, I. A. Ojo, A. C. Adebajo et al., **2012,** *Advances in Biological Chemistry*, vol. 2, pp. 268–273.
[33]. I. Bertini, H. B. Gray, E. I. Stiefel, J. S. Valentine, *Biological Inorganic Chemistry: Structure and Reactivity*, University Science Books, Sausalito, Calif, USA, 1st edition, 2007.
[34]. M. Carcelli, P. Mazza, C. Pelizzi, G. Pelizzi, F. Zani, *J. Inorg. Biochem.*, **1995**, 57, 43.