

FULL LENGTH RESEARCH ARTICLE

SYNTHESES OF COPPER COMPLEXES OF NICOTINOHYDROXAMIC
AND ISONICOTINOHYDROXAMIC ACIDS.

*ALIYU, A. O.¹; EGWAIKHIDE, A. P.² & GIMBA, C. E.³

¹Department of Chemistry

Nigerian Defence Academy, Kaduna, Nigeria.

²Department of Chemistry and Centre for Biomaterials Research

University of Benin, Nigeria

³Department of Chemistry

Ahmadu Bello University, Zaria Nigeria

*(Corresponding author)

pegwaikhide@yahoo.com

ABSTRACT

Nicotinohydroxamic acid (NHA) and isonicotinohydroxamic acid (INHA) were synthesized, characterized by electronic and spectral studies, magnetic measurements and their pKa determined spectrophotometrically as 8.68 ± 0.02 in aqueous medium of 0.1 mol dm^{-3} ionic strength. The composition of the complexes was determined by Job's plot. The ratios of Cu^{2+} to ligands under investigation were ML_2 . The formation constants obtained and the possible binding modes for the complexes in solid states are discussed. Spectral studies of the isolated complexes indicate tetragonally distorted octahedral geometry via (O,O) and (N,O) coordination modes. The magnetic moments obtained for the complexes are in the range 1.57-1.79 B.M. Microbial sensitivity test carried out on the ligands and their isolated complexes showed no activity on the microorganisms under investigation.

Keywords: Nicotinohydroxamic acid, isonicotinohydroxamic acid, IR spectra, ionic strength, Job's plot, pKa, microbial sensitivity.

INTRODUCTION

Hydroxamic acids have general formula RCONHOH . These acids are much weaker acids than the structurally related carboxylic acids RCOOH (Celine 2000). Hydroxamic acids are ubiquitous in nature and are associated with iron transport bacteria (Nwabueze 1996). The selectivity of the mechanism of iron transport phenomena is important since other metal ions, which may be essential or toxic to the organism are present in the environment (Kehl 1982; Raymond 1990 and Crumbliss 1991). Hydroxamic acids with one or more $-\text{CONHOH}$ groups have been extensively studied in relation to their pharmacological, toxicological and pathological properties (Paniago & Carvalho 1988; McLachlan *et al.* 1983; Fatima *et al.* 2002) which is related with their ability to form metal ion complexes. Medical applications of the hydroxamates which utilize their affinity for high charge density metal ion include the possible use of the metal complexes as imaging agents (Biljana *et al.* 2002; Hirsova & Koldovish 1969). Hydroxamic acids are constituent of antibiotics, growth factors, food additives, tumor inhibitors and cell division factors (Albrecht-Gary & Crumbliss 1981; Hartley *et al.* 1980; Martell *et al.* 1981).

With regards to the strong ability of the hydroxamic acids to form chelates, clarification of their interactions with metal ions of particular biological effect is necessary. In the present study, equilibrium and structural studies have been performed on the copper (II) complexes of nicotinohydroxamic acid and isonicotinohydroxamic acid.

MATERIALS AND METHODS

Ethyl nicotinate and ethyl isonicotinate were used as purchased without further purification. Water was doubly distilled and degassed using purified N_2 . All other reagents were used as supplied. Radiometer Copenhagen Research pH meter was used for pH measurement. IR spectra were recorded on ATI Maltson Genesis series FTR™ machine

as Nujol mulls in the $4000\text{-}200\text{cm}^{-1}$ spectra region. MSB AUTO magnetic susceptibility balance was used to measure room temperature magnetic susceptibility.

Nicotinohydroxamic acid was prepared by adding 2.3g sodium metal in 50cm^3 to 6.9g NH_2OH . HCl dissolved in 100cm^3 MeOH. The mixture was cooled to room temperature and 15.12g ethyl nicotinate was added. The mixture was stirred for 40min. and another solution of 2.3g Na in MeOH was added and stirring was continued for another 10min. the mixture was filtered to remove the precipitated NaCl and the filtrate acidified with concentrated HCl. The filtrate was concentrated using a rotary evaporator and left in a refrigerator to crystallize. The crystals were removed by filtration and recrystallized from EtOH with 55% yield. Similarly, isonicotinohydroxamic acid was prepared by using 15.12g ethyl isonicotinate 2.3g Na metal in 50cm^3 of MeOH respectively.

$[\text{Cu}(\text{NHA})_2] \cdot 2\text{H}_2\text{O}$ and $[\text{Cu}(\text{INHA})_2] \cdot 2\text{H}_2\text{O}$ were prepared using 0.556g NHA and INHA in 20cm^3 of MeOH added to 0.5g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in cold water. The mixture was allowed to stay for 2hr to allow the precipitate to settle. A green coloured precipitate was removed by filtration, washed with small aliquots of Et_2O and dried over silica gel in a vacuum desiccator.

Equilibrium Studies: The pKa values for the ligands were determined spectrophotometrically as described by Albert and Sergent (1971) using boric acid and borax of ionic strength 0.1 mol dm^{-3} and $0.025 \text{ mol dm}^{-3}$ buffers for NHA and INHA ligands (Aliyu *et al.* 2008).

Antimicrobial screening: All media and bacterial suspensions were prepared as described by Cruickshank (1965). The antimicrobial

activity of the test compounds was assayed against six bacterial strains of three Gram + ve and three Gram-ve (Aliyu *et al.* 2008).

RESULTS AND DISCUSSION

The high basicity of the ligands under study may be ascribed to the positive inductive effect of the bulky pyridine group attached to the

functional groups of NHA and INHA respectively. The pKa values of the ligands are 8.68 ± 0.02 for NHA and 8.68 ± 0.05 for INHA. The absorption spectra of solutions containing a constant metal concentration but variable ligand molar concentrations of NHA and INHA are shown in Figs 1 and 2 While graphical matrix rank analysis of the absorbance data generated from similar solution for NHA and INHA are indicated in figures 3 and 4.

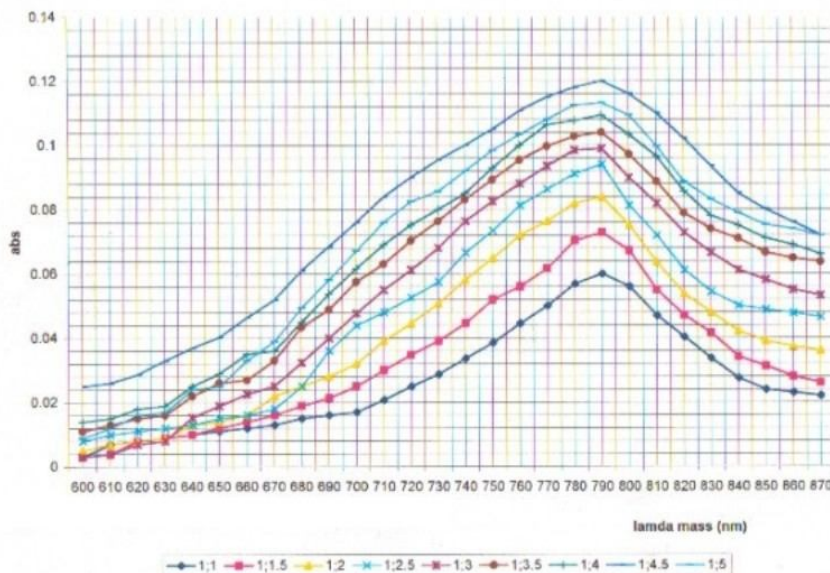


FIG 1: SPECTRA OF SOLUTION OF DIFFERENT M.L RATIOS FOR THE $\text{Cu}^{2+}/\text{NHA}$ SYSTEM SHOWING THE ABSENCE OF ISOSBESTIC POINTS

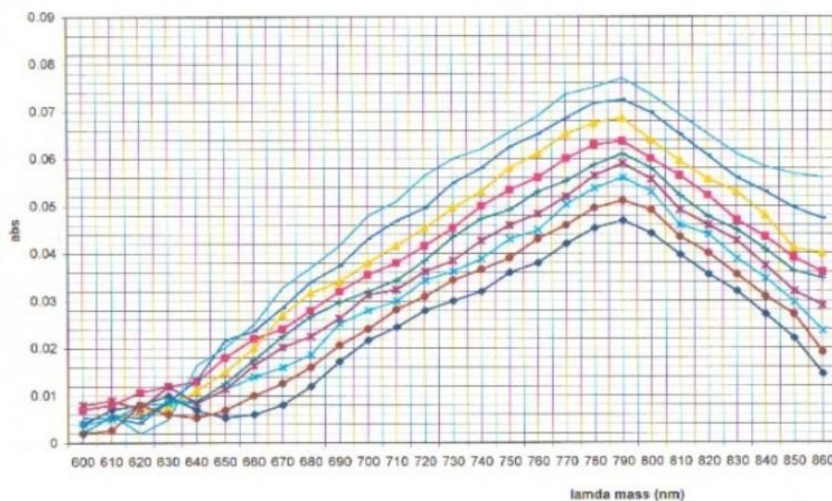


FIG 2: SPECTRA OF SOLUTION OF DIFFERENT M.L RATIOS FOR THE $\text{Cu}^{2+}/\text{INHA}$ SYSTEM SHOWING THE ABSENCE OF ISOSBESTIC POINTS.

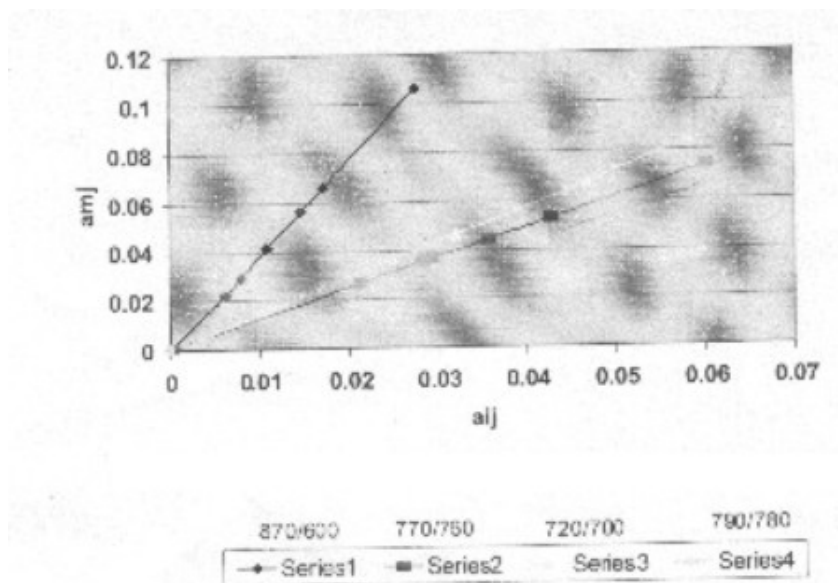


FIG 3: GRAPHICAL RANK MATRIX ANALYSIS Cu^{II} - NHA SYSTEM (ONE SPECIE TEST)

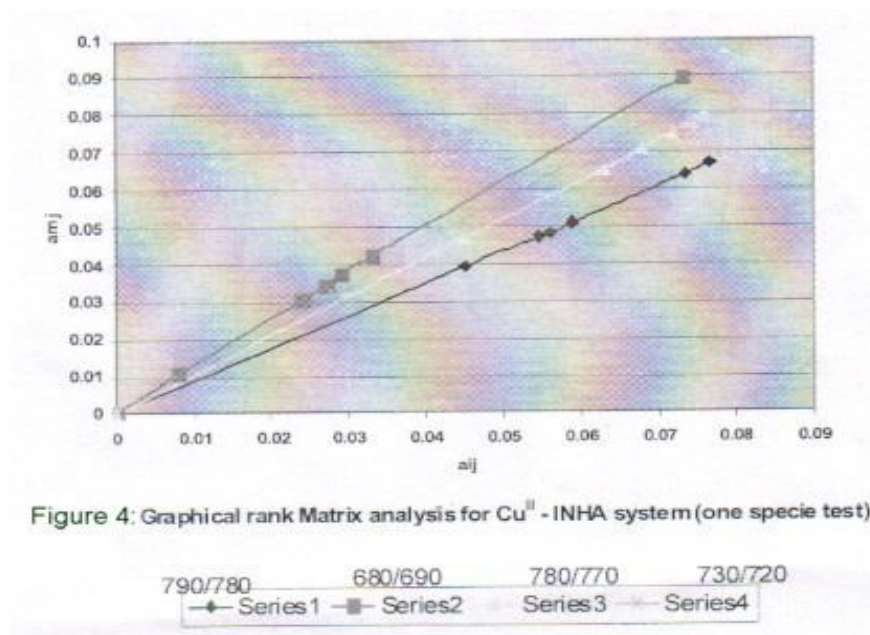


Figure 4: Graphical rank Matrix analysis for Cu^{II} - INHA system (one specie test)

The shape of the graphs (Figs 1 and 2) and the absence of an isobestic point are typical of systems containing only one complex specie (Hartley *et al.* 1980).

Several equilibrium models were tried but it was only in ML_2 model that convergence was achieved. The complex composition was determined by Job's plot as shown in figures 5 and 6.

The ratios of Cu^{II} - to the ligands under investigation were ML_2 . Table 1 gives the analytical data and some physico-chemical properties of copper (II) complexes. The observed magnetic moments at room temperature were between 1.57 and 1.79 MB thus ruling out the possibility of Cu - Cu interaction in these complexes (Nicholls 1979). The range of magnetic moments is irrespective of the stereochemistry. The visible spectra of copper(II) hydroxamate complexes, ranges between 630 - 810 nm as shown in Figs 7 and 8.

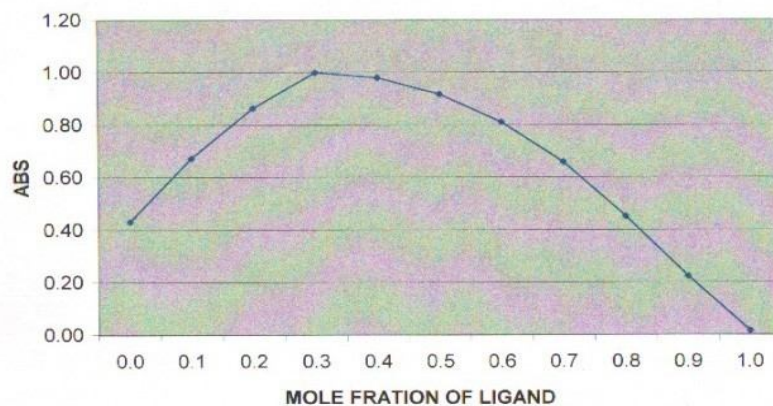


FIG 5: CONTINUOUS VARIATION (JOBS PLOT) METHOD $\text{Cu}^{2+}/\text{NHA}$ SYSTEM

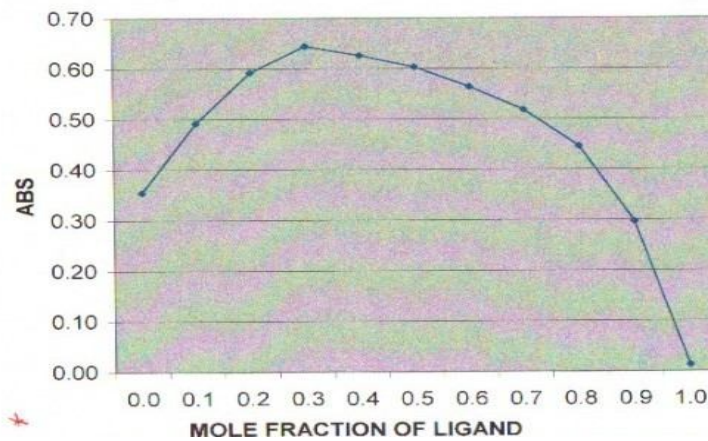


FIG 6: CONTINUOUS VARIATION (JOBS PLOT) METHOD $\text{Cu}^{2+}/\text{INHA}$ SYSTEM

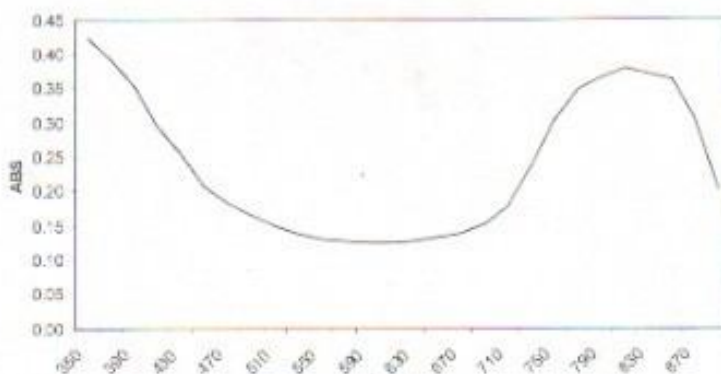


FIG 7: VISIBLE SPECTRUM OF $[\text{Cu}(\text{NHA})_2] \cdot 2\text{H}_2\text{O}$ COMPLEX.

These bands are assigned d→d transitions of copper (II) ions and encompasses several over-lapping bands. The electronic spectra of Cu^{II} – NHA and Cu^{II}-INHA (Figs. 7 and 8) do not resemble the spectra of standard square planar copper (II) complexes but more closely agree with the spectra of established tetragonally distorted octahedral complexes. The range of standard square planar copper (II) complexes is between 714 – 500nm, (Nichlol 1979; Cotton & Wilkinson 1980).

Table 2 shows the diagnostic IR band of the metal free ligands and their corresponding complexes. In the spectra of the metal complexes, the observed band in the region of 3374.43 cm⁻¹ and 3420 cm⁻¹ were assigned to ν(NH) stretching vibration in Cu^{II} – NHA and Cu^{II} – INHA respectively. The observed decrease in the frequency of this band is about 44cm⁻¹ relative to the position in the metal free ligand and this is due to the deprotonation of the nitrogen atom of the hydroxamate group thereby indicating complexation through the nitrogen atom (Cu^{II} – NHA).

But in the Cu^{II} – INHA complex, there was little or no increase in the observed frequency relative to its metal free ligand implying the absence of coordination through the nitrogen atom.

The band around 1605.40cm⁻¹ and 1561.58cm⁻¹ in the spectra of copper (II) hydroxamate complexes were assigned to the ketonic carbonyl frequencies. The decrease in the frequencies to about 54.21 cm⁻¹ and 43.43 cm⁻¹ respectively relative to the position of the metal free ligand suggests of coordination through the ketonic carbonyl oxygen of the hydroxamate group (Biljana *et al.* 2002; Chatterjee 1978; West 1969). The V(CN) frequencies were observed around 1112.09cm⁻¹ and 1129cm⁻¹ for Cu^{II} – NHA and Cu^{II} – INHA respectively. The observed increase in the frequencies relative to their metal free ligand is expected. Based on the IR data therefore, the following bonding modes were suggested for the copper (II) hydroxamate complexes. Cu^{II} – NHA bonding mode is (N, O) while Cu^{II}-INHA bonding is (O, O) as suggested.

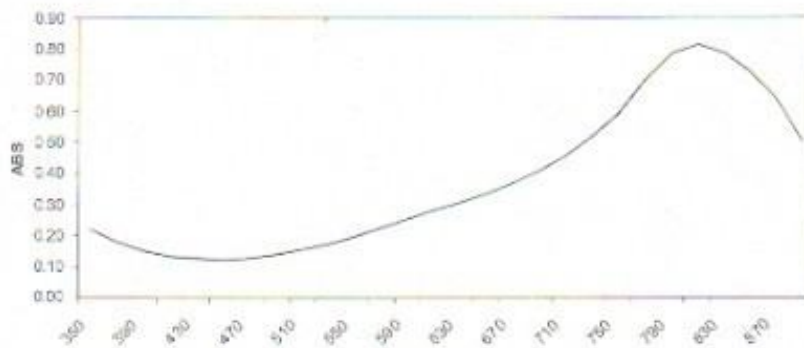


FIG 8: VISIBLE SPECTRUM OF [CU (INHA)₂] 2H₂O COMPLEX

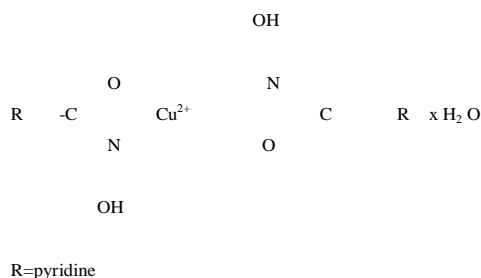


FIG 9. SUGGESTED STRUCTURE FOR THE (N, O) BONDING MODE FOR TETRAHEDRALLY DISTORTED OCTAHEDRON COORDINATED COMPLEX OF COPPER (II) HYDROXAMATE.

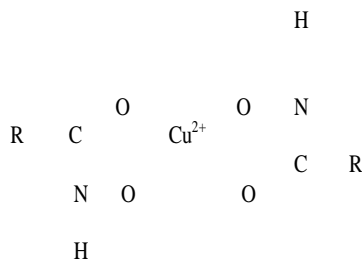


FIG10; SUGGESTED STRUCTURE FOR (O, O) BONDING FOR SQUARE PLANAR COMPLEXES OF COPPER (II) HYDROXAMATE.

The microbial sensitivity tests carried out on the ligands and the copper (II) complexes show no activity on the micro-organism under investigation as shown in Table 3.

TABLE 1: ANALYTICAL DATA AND PHYSICO-CHEMICAL PROPERTIES FOR THE ISOLATED COMPLEXES (CALCULATED%)

Compound	Formular weight	Melting point/Decomposition point °C	Found Metal%	μ_{eff} Borh Mangeton at 298k	λ_{max} (nm)	Colour	Assignment
Cu(NHA) ₂ .2H ₂ O	375.5	269	16.82(16.91)	1.57	800	Light green	d→ d
Cu(INHA) ₂ .2H ₂ O	375.5	276	16.82(16.91)	1.79	800	Green	d→ d

TABLE 2. DIAGONISTIC IR DATA FOR THE COMPLEXES (CM⁻¹)

Compound	V(NH)	Δ V(NH)cm ⁻¹	V(C = O)cm ⁻¹	Δ V(C = O) cm ⁻¹
NHA	3418.00		1659.61	
Cu(NHA) ₂ .2H ₂ O	3374.43	- 44.00	1605.40	-54.21
INHA	3422.59		1605.01	
Cu(INHA) ₂ .2H ₂ O	3420.00	- 2.59	1561.58	-43.43

Key:
NHA: Nicotinohydroxamic acid
INHA: Isonicotinohydroxamic acid

TABLE 3: MICROBIAL SENSITIVITY TEST FOR THE LIGANDS AND THEIR COPPER (II) COMPLEXES

Ligands/complexes	<i>S. aureus</i>	<i>S. typhium</i>	<i>E. coli</i>	α -heamolytic strep	<i>Klebsiella</i>	<i>Pseudomonas</i>
NHA	-	-	-	-	-	-
Cu(NHA) ₂ .2H ₂ O	-	-	-	-	-	-
INHA	-	-	-	-	-	-
Cu(INHA) ₂ . 2H ₂ O	-	-	-	-	-	-

Key – not present

The apparent drug resistance exhibited by the four species of gram-ve and gram+ve bacterial strains tested during this study suggests these could be nosocomial (hospital) microorganism. Genetically developed multi-drug resistance mechanisms could have arisen in these microbial stains as a result of their rampant exposure to several antibiotics, characteristic of hospital environment.

These mechanisms include non permeability of the microbial outer membrane to chemical bactericides, development of multidrug resistant pump mechanisms that expel absorbed drugs by microbes, inactivation of absorbed drugs by antimicrobials through their chemical modification, bypassing of metabolic sequence inhibited by drugs and increase in the production of metabolite target of antimicrobial agents (Prescott *et al.* 1999).

Albert, A. & Serjeant, E. P. 1971. *The determination of ionization constant*. 2nd Edition p 44. Chapman and Hall. Ltd. London

Albrecht-Gary A. M, & Crumbliss A. L. 1998. *Metal Ions in Biological systems*. Vol. 35 Marcel Delker, New York.

Aliyu, A. O., Egwaikhide, P. A Maikaye, D. B & Gimba, C. E. 2008. Complex formation between transition metals. *Pakistan Journal of scientific and industrial research*, 2(1):213-219.

Biljana, N., Nikola, K., Krisimir, S., & Drazen V. T. 2002. Complex formation between transition metals and 2 – pyrrolidine- 5 – hydroxamic acid. *Acta Chemistry Slovenia* 49:325-535.

Celine, J. M. & Kelvin, B. N. 2000. Hydroxamic acids-ion chelators, aspirin analogues, nitric oxide donors and structurally diversified metals complexes. <http://www.irishscientist>.

Chatterje, B. 1978. *Coordination Chemistry, Revision*. Elsevier Science limited London

Cotton, F.A. & Wilkinson, G. 1980. *Advance Inorganic Chemistry Comprehensive test* 4th Edition, Wiley, New York

Cruickshark, R. 1965. *Medical micro-biology*. 2nd Edition. Church and Livingstone. U.K

Crumbliss, A. L. 1991. *Handbook of microbial iron chelate*. 2nd Edition G. Winkelmann CRC. Press. New York.

Fatima, N, Maqsood, Z. T. & Kazmi, S. A. 2004. Complexation of vanadium (iv) with hydroamate chelators and Their stability relation with pH. *Journal of Chemical Society of Pakistan* 24:49

Hartlev, F. R.; Burdass, C. & Alcocook, R. M. 1980. *Solution*

Kehl, H. 1982. *Chemistry and Biology of hydroxamic acids*. Karger, New York.

Nicholl, D. 1979. *Complexes and first row transition elements*. Macmillan Press Ltd. London.

Martell, A. E.; Anderson, W. F. & Badman, D. G. 1981. *Development of iron Chelators for Clinical use*. Elsevier. New York

Mclachlan, D. R. C.; Faenell, B.; Gallin, H.; Karlik, S.; Eichorn, G. & DeBoni, U. 1983. *Biological Aspects of metal and metal – related diseases*. Raven Press. New York

Nwabueze, J. N. 1996. Complexes iron(III) with cyclopropane carbo-and cyclohexyl acetohydroxamic acids. *Transition metal chemistry*. Vol. 21 pp 258 – 261. Thermochemical acta, Elsevier London.

Paniago, E. B & Carvalho, S. 1988. *Ciencia e Cutura* 40 pp 629. University of Mato Grosso do Sul (Hu – UFMS)

Prescott, L. M.; Harley, J. P. & Klein D. A. 1999. *Antimicrobial Chemotherapy. Drug resistance in Microbiology* 4th Edition pp 690. WCB/MCGram Hill.

West, T. S. 1969. *Complexometry with EDTA and related reagents*. BDH Chemicals Ltd London.