Biological screening and spectral study of six co-ordinate octahydral complexes of Fe (III) of hydrazide

Jayesh. R. Gujarathi[1], Professor
School of Chemical Sciences,Pratap college Amalner (Autonomous)
Dist-Jalgaon (M.S.) India
Tushar V.Rajale[2],Associate Professor
School of Chemical Sciences,Pratap college Amalner (Autonomous)
Dist-Jalgaon (M.S.) India
Dr.Garima Sharma, Professor of Chemistry and
Deputy -Director Research, Motherhood University,Roorkee

Abstract :-Hydrazide ligands were prepared using acetohydrazide with 3,5-dichloro-2-hydroxy acetophenone, 5-chloro-2-hydroxy acetophenone and 4,5-dichloro-2-hydroxy acetophenone.Its complexes with Fe (III) were synthesized by reaction of $FeCl_3.6H_2O$ with hydrazide lignds in mole ratio 1;2.The ligands and complexes were characterized by Elemental analysis,ESI-MS,Infrared (FT-IR) spectroscopy,electronic spectra,Nuclear Magnetic Resonance (1HNMR and ^{13}CNMR) magnetic measurement and conductivity at different temperature.The metal complexes and corresponding ligands were screened against bacterial species. It has been observed that the Fe (III) complexes had shown more activity than corresponding hydrazide ligands.

Keywords: 1:2 ratio,bioactive metal complexes,paramagnetism,$FeCl_3$.6H2O.

INTRODUCTION

Hydrazide complexes possess biological properties such as antibacterial ,antifungal ,anti-tumor ,anti-malarial ,anti-cancer ,anti-inflammatory [1-11]. Schiff bases form stable complexes with most transition metal ions,hence they play important role in Inorganic Chemistry.Schiff base complexes are proved as biologically important species [12-16]The remarkable biological activity of acid hydrazides, a class of Schiff base, their corresponding aroylhydrazones, and the dependence of their mode of chelation with transition metal ions present in the living system have been of significant interest[17-23]. Isonicotinic acid hydrazide is a drug of proven therapeutic importance and is used as in bacterial diseases, e.g., tuberculosis [24]. The transition metal complexes of Schiff base ligands are important , not only due to their spectroscopic properties and applications [25] but also due to their antifungal, antibacterial and antitumor activities [26]. Schiff bases have a vital position in metal coordination chemistry even almost a century since their discovery. Large number of metal coordination complexes of Schiff bases have been used as antibacterial, antifungal, cytotoxic, anti-inflammatory and cytostatic agents [27-30].

In this research tenure the synthesis, spectral characterization,conductivity measurement and biological screening of six coordinate complexes of Fe (III) with hydrazide ligands have been reported.

II MATERIALS AND METHODS

A.R.grade chemicals were used. Magnetic susceptibility measurement was carried out by Faraday method at room temperature. Infra red spectra (Refluctance spectra) were recorded in solid state in the range 4000-200 cm^{-1} range.Thermogravimetric anaysis was carried out in the temperature range 30-800^{o}C.Metal was estimated using standardized E.D.T.A ,diphenyl amine as an indicator and PH-10 buffer solution .

III EXPERIMENTAL

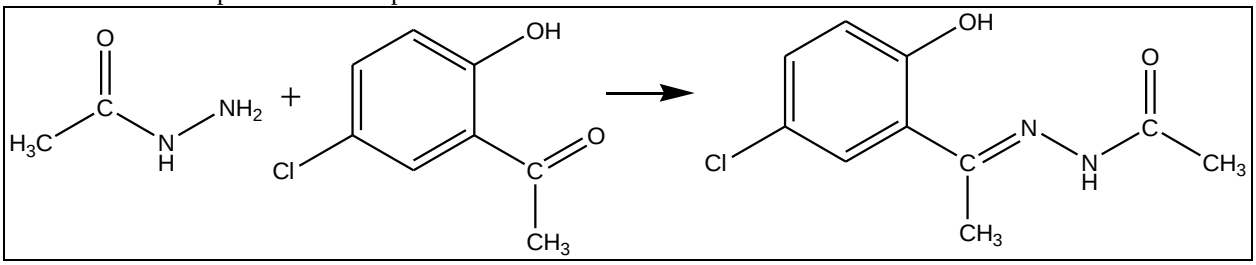
Synthesis of acetohydride:

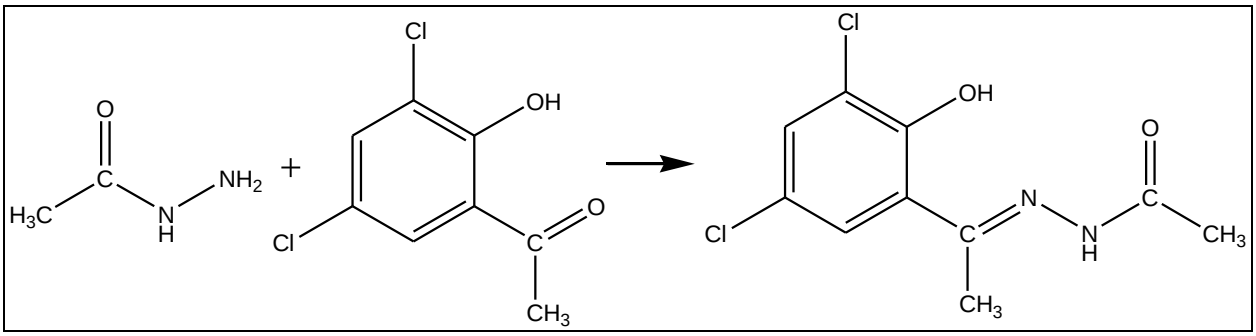
20 ml ethanolic solution of glacial cetic acid (0.01 mole) was added to 20 ml ethanolic solution of hydrazine hydrate (0.01 mole) in the mole ratio 1:1.The reaction mixture was refluxed for three hours. On cooling pale yellow product was filtered and washed with hot water then cold ethanol and finally with ether. The compound was then purified in ethanol and dried in vacuum.

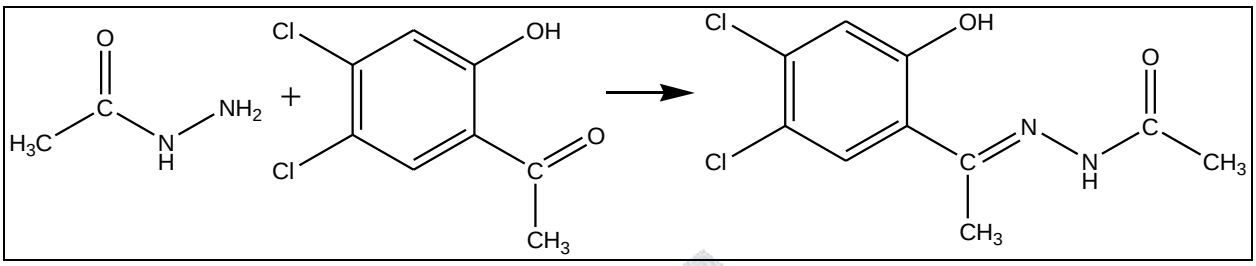
$$CH_3COOH + NH_2.NH_2.H_2O \rightarrow CH_3CONHNH_2 + H_2O$$

Scheme I

20 ml ethanolic solution of acetohydride (0.01 mole) was added to 20 ml ethanolic solution 5- chloro 2-hydroxy acetophenone/3,5 dichloro 2-hydroxy acetophenone/4,5 dichloro 2-hydroxy acetophenone (0.01 mole) in the mole ratio 1:1.The reaction mixture was refluxed for three hours. On cooling pale yellow product was filtered and washed with hot water then cold ethanol and finally with ether. The compound was then purified in ethanol and dried in vacuum.







Synthesis of complex

The complexes were synthesized by adding slowly ethanolic solution of $FeCl_3.6H_2O$ (0.01 mole) to the hot ehanolic solution of 5-chloro 2-hydroxy acetophenone acetohydride/3,5-dichloro 2-hydroxy acetophenone acetohydride/4,5-dichloro 2-hydroxy acetophenone acetohydride (0.01 mole) in the ratio 1:2 and starring reaction mixture for half hour at 30^0C temperature. The complex obtained was filtered and washed with hot water to remove excess metal salt, cold ethanol and diethyl ether and dried in vacuum.

Physical measurements

NMR spectra were recorded in the mixture of $CDCl_3$ and DMSO-d_6 (1:1 v/v) with a Bruker AC-300F 300MHz spectrometer.

Table 1 Physical measurements

Compounds	Colour	Empirical Formula	Molar conductance Ohm^{-1}cm^{2}mole^{-1}	Magnetic Moment B.M.
L	Yellow	$C_{10}H_{11}N_2O_2SCl$	-	-
L’	Yellow	$C_{10}H_{10}O_2N_2Cl_2$		
L’’	Yellow	$C_{10}H_{10}O_2N_2Cl_2$		
Fe-L_2	Dark brown	$C_{20}H_{24}N_4O_6Cl_2Fe$	45.5	5.80
Fe-L_2’	Dark brown	$C_{20}H_{22}N_4O_6Cl_4Fe$	53.3	5.77
Fe-L_2’’	Dark brown	$C_{20}H_{22}N_4O_6Cl_4Fe$	54.8	5.78

Conductivity measurement

The complexes are soluble in DMF so conductivity measurement measured using DMF solutions and equivalent conductance calculated. 0.001 M solutions of complexes were prepared in different percentages of DMF-ethanol mixture and the parameter of solution under study was calculated at temperature 305 K, 310 K and 315 K.

Equivalent conductance at 300 K			
DMF-Ethanol mixture	Fe.L_2.	Fe.L_2’	Fe.L_2’’
75%	35.2	36.7	36.4
80%	36.3	36.0	38.6
85%	47.5	43.0	40.5
90%	48.6	45.0	43.2
95%	51.7	49.3	47.6
100%	54.8	51.8	50.4
Equivalent conductance at 305 K			
DMF-Ethanol mixture	Fe.L_2.	Fe.L_2’	Fe.L_2’’

75%	41.9	39.5	40.1
80%	44.6	40.2	43.4
85%	47.4	44.2	45.4
90%	53.6	49.3	47.2
95%	56.4	51.4	49.7
100%	59.8	55.3	52.4

Equivalent conductance at 310 K			
DMF-Ethanol mixture	$Fe.L_2$.	$Fe.L_2$'	$Fe.L_2$''
75%	43.7	40.3	40.5
80%	45.8	42.6	43.4
85%	47.6	44.4	51.3
90%	51.4	51.4	50.3
95%	56.2	52.5	53.6
100%	62.5	56.6	61.2

^{1}HNMR (L)

NMR signals at 10.00 and 2.02 ppm and 0.9 ppm are assigned to – OH and O=C-CH_3 and–N=C-CH_3 protons respectively. Signal at 7.0 ppm corresponds to NH. Aromatic protons show multiplets at 7.1, 7.5, 6.7, ppm.

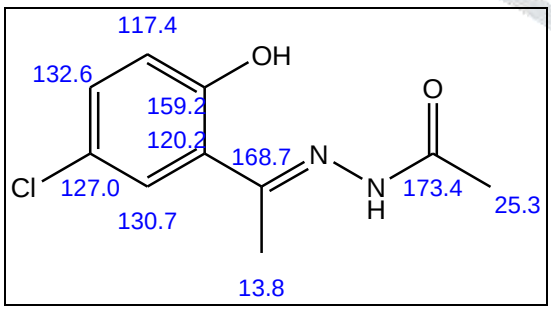
^{1}H-NMR (L')

NMR signals at 10.00 and 2.02 ppm and 0.9 ppm are assigned to – OH and O=C-CH_3 and–N=C-CH_3 protons respectively. Signal at 7.0 ppm corresponds to NH. Aromatic protons show multiplets at 7.1, 7.3 ppm.

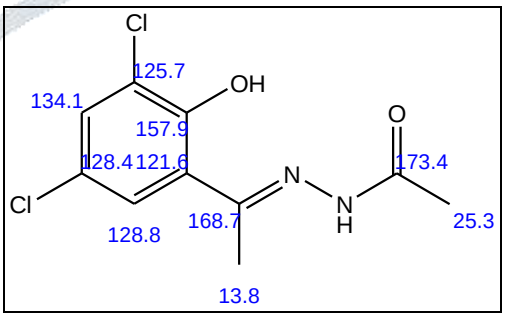
^{1}H-NMR (L'')

NMR signals at 10.00 and 2.02 ppm and 0.9 ppm are assigned to – OH and O=C-CH_3 and–N=C-CH_3 protons respectively. Signal at 7.0 ppm corresponds to NH. Aromatic protons show multiplets at 7.4, 6.7 ppm.

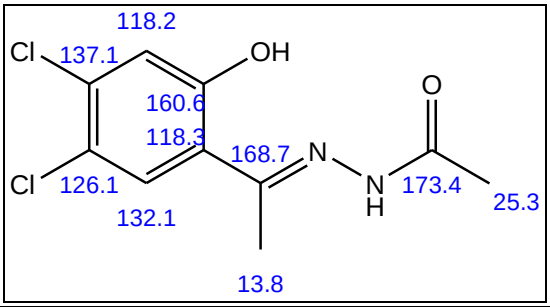
^{13}C-NMR (L) δppm



^{13}C-NMR (L') δppm



^{13}C-NMR (L'') δppm



ESI-MS m/z, ion M^+(Calcd) found

$C_{10}H_{11}N_2O_2SCl$ (226.65) 226.10, $C_{10}H_{10}O_2N_2Cl_2$ (261.09) 261.91, $C_{10}H_{10}O_2N_2Cl_2$ (261.09) 261.60, $C_{20}H_{20}N_4O_4Cl_2Fe$ (507..28) 507.82, $C_{20}H_{18}N_4O_4Cl_4Fe$ (576.17) 576.74, $C_{20}H_{18}N_4O_4Cl_4Fe$ (576.17) 576.75.

Table.2 Analytical data

Compounds	Elemental Analysis Found (Calculated) %				
	Metal%	%C	%H	%N	%O
L	-	52.11 (52.99)	4.22 (4.89)	12.71 (12.36)	14.84 (14.12)
L’	-	46.71 (46.00)	3.12 (3.86)	10.09 (10.73)	12.72 (12.27)
L’’	-	46.85 (46.00)	3.04 (3.86)	10.11 (10.73)	12.79 (12.27)
$Fe.L_2$	11.84 (11.04)	47.91 (47.35)	3.12 (3.97)	11.94 (11.04)	12.01 (12.62)
$Fe.L'_2$	9.07 (9.72)	41.09 (41.69)	3.84 (3.15)	9.13 (9.72)	11.82 (11.11)
$Fe.L''_2$	9.12 (9.72)	41.12 (41.69)	3.95 (3.15)	9.10 (9.72)	11.75 (11.11)

Electronic Spectral data (cm^{-1}) in solid state

Table 3 .Electronic spectral data (cm^{-1})

Compound	d-d	L→M	n→π*	π→π*
L	-	-	25,971	40,865
L’	-	-	25,880	40,200
L’’	-	-	25,350	40,140
$Fe.L_2$	17,500	23,700	33,200	42,250
$Fe.L_2$’	17,750	23,600	33,500	42,300
$Fe.L_2$’’	17,800	23,950	33,400	42,950

Infrared Spectroscopic data (cm^{-1})

IR-spectral data

1.L: ν (- OH) 3200; ν (C = N) 1670,ν (N – N) 1050; ν (^{2}N-H) 3250; ν (C – O) 1290.

2.L’: ν (- OH) 3260; ν (C = N) 1675,ν (N – N) 1075; ν (^{2}N-H) 3255; ν (C – O) 1285.

3.L’’: ν (- OH) 3299; ν (C = N) 1685,ν (N – N) 1080; ν (^{2}N-H) 3260; ν (C – O) 1288.

4.$Fe.L_2$: ν (- OH) 3225,ν (C = N) 1569,ν (N-N) 1165, ν (N-H) 3260ν (Fe- N) 450, ν (Fe - O) 545,ν (C - O) 1218

5.$Fe.L_2$': ν (- OH) 3265,ν (C = N) 1573,ν (N-N) 1178, ν (N-H) 3270ν (Fe - N) 460, ν (Fe - O) 550,ν (C - O) 1228.

6.$Fe.L_2$'': ν (- OH) 3285,ν (C = N) 1578,ν (N-N) 1188, ν (N-H) 3270ν (Fe - N) 470, ν (Fe - O) 560,ν (C - O) 1235.

TGA ANALYSIS DATA:

The TGA was carried out in the temperature range 25 °C to 800 °C

1.**$Fe.L_2$**: First H_2O 110^{0}C Mass loss 3.80, Second H_2O 112^{0}C Mass loss 6.33 First step, 115 °C, Mass loss 4.70 % second step, 143.0 °C, Mass loss, 20.30 % Third Step 255.0 °C, Mass loss, 40.0 % Fourth Step, 375.0 °C, Mass loss .70.0 %, Residue 800 °C, % of Fe_2O_3, 31.97(31.51).

2.**$Fe.L_2$'**: First H_2O 109^{0}C Mass loss 2.52, Second H_2O 113^{0}C Mass loss 5.28, First step, 125 °C, Mass loss 11.58 % second step,366.0 °C, Third Step 245.0 °C, Mass loss, 42.60 % Fourth Step, 385.0 °C, Mass loss .70.0 %, Mass loss, 70.0 % , Residue, 785 °C, % of Fe_2O_3, 27.11 (27.74).

3.**Fe-L_2''.** : First H_2O 108^{0}C Mass loss 2.30, Second H_2O 115^{0}C Mass loss 5.30,First step, 124 °C, Mass loss 11.75 % second step 365 °C, Mass loss, 64.0 % , Third Step 260.0 °C, Mass loss, 48.02 % Fourth Step, 375.0 °C, Mass loss .70.0 %, Residue 780 °C, % of Fe_2O_3, 27.20 (27.74).

IX BIOLOGICAL ACTIVITY (AGAR PLATE DIFFUSION METHOD)

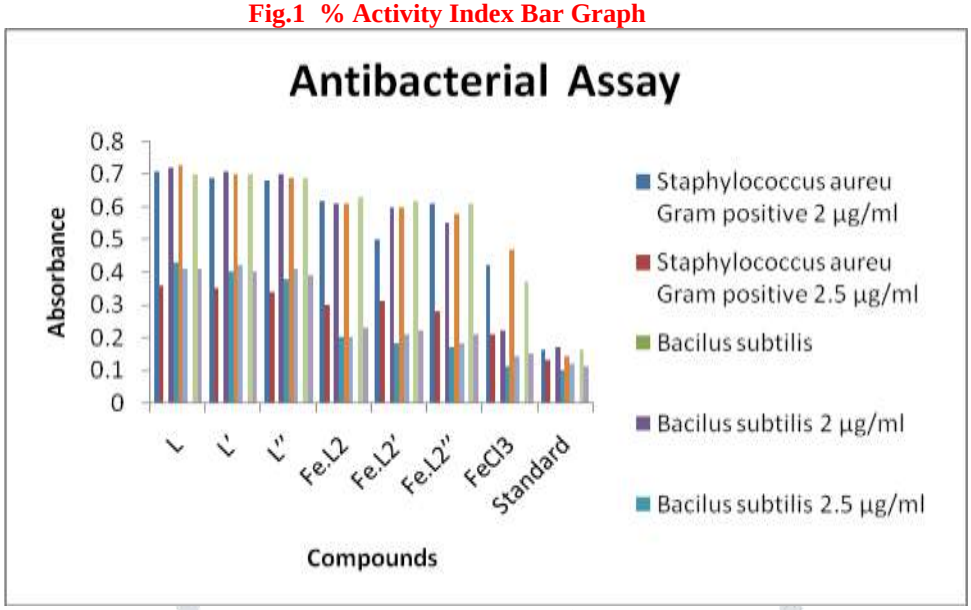
Table.4 Minimum Inhibitory concentration L, Fe (III) complexes and standered

Compound	***Staphylococcus aureu***		***Bacilus subtilis***		***Escherichia Coli***		***Peudomonas aeruginosa***	
	Gram positive				Gram negative			
	2 µg/ml	2.5 µg/ml	2 µg/ml	2.5 µg/ml	2 µg/ml	2.5 µg/ml	2 µg/ml	2.5 µg/ml
L	0.71	0.36	0.72	0.43	0.73	0.41	0.70	0.41
L’	0.69	0.35	0.71	0.40	0.70	0.42	0.70	0.40
L’’	0.68	0.34	0.70	0.38	0.69	0.41	0.69	0.39

$Fe.L_2$	0.62	0.30	0.61	0.20	0.61	0.20	0.63	0.23
$Fe.L_2$'	0.50	0.31	0.60	0.18	0.60	0.21	0.62	0.22
$Fe.L_2$''	0.61	0.28	0.55	0.17	0.58	0.18	0.61	0.21
$FeCl_3$	0.42	0.21	0.22	0.11	0.47	0.14	0.37	0.15
Standard	0.16	0.13	0.17	0.10	0.14	0.12	0.16	0.11

(Std-Amphiciline)

Fig.1 % Activity Index Bar Graph



RESULTS AND DISCUSSION

Metal ion and ligand in all complexes found 1:2.The complexes are insoluble in DMF solvent.The conductivity measurements were carried out in DMF.All complexes showed electrolyte behaviour [31].Mass spectral data confirmed the structure of ligands and complexes. The ground term is ^6S in high-spin complexes.For the ^6A_1g ground term in an octahedral; field there is no reduction of the moment below the spin-only value by spin -orbit coupling with higher liquid field terms.The magnetic moments are found to be very close to the spin-only value of 5.92 B.M.and to be independent of temperature.In the octahedral complexes with $^6A_1$ ground term also shows the similar behavior.A comparison of the variation of moment with temperature gives an estimation of the extent of electron delocalization and the magnitude of the low symmetry liquid field component.The magnetic susceptibility data of the Fe (III) complexes of ligands studied in the present work are listed in Table No.1.The magnetic susceptibility measurements of the complexes showed that complexes are paramagnetic in nature.

Different concentration of solutions were prepared and equivalent conductance of solutions was measured as it depaends on concentration and temperature.It has been found that equivalent conductance of an electrolyte increases with increase in dilution. In dilute solution conductance increases.Equivalent conductance increases with dilution at 305 K, 310K,315 K.The conductivity of an electrolyte depends upon the temperature. The Equivalent conductance of an electrolyte increases with temperature. This is because at higher temperature the mobility of ions increases and hence the conductivity.

The electronic spectra showed band in 40,000-41,000 cm^{-1} range and 25,000 – 26,000 cm^{-1} range, these can be assigned to Π – Π* (aromatic ring) and n – Π* transitions respectively.The broad bands in 33,000 – 34,000 range are assigned for n – $Π^*$ transitions in all complexes [32]. The shift of Π – Π* bands are shifted to the longer wavelength region in complexes.The spectra show d–d spectral transitions in the range17,000 – 18,000 cm^{-1}The bands at 25,000 – 26,000 cm^{-1} range correspond to L → M.

.These bands suggested octahedral geometry around Fe(III) [33].

The coordination due to azomethine nitrogen shifted ν(C = N) shifted to lower wavenumbers and ν(N-N) shifted to higher wavenumbers confirm coordination of azomethine nitrogen [34].The band at 450 – 470 $cm^{-1}$is assignable to ν(Fe– N) confirmed the coordination of azomethine nitrogen.The bands at 545–560 cm^{-1} is assignable to ν(Fe-O). The band due to N-H in in the complexes is not affected.The band due to OH in in the complexes is not affected.

Co-ordinated water molecules were removed at a temperature about 108-110°C corresponding to mass loss 2.0-4.0 %. Decomposition proceeded in certain steps .There was no change observed up to~200 °C after that break in the curves due to evaporation of part of molecule of organic ligand, the remaining hydrazine molecule was removed from the coordination sphere at ~600°C.The metal oxides were formed above 700°C.The decomposition was completed at ~800 °C.It has been found that Fe (III) complexes are stable up to 200°C and decomposition took place above this temperature and completed in the temperature range 300-400°C. The second steps are in the range of 310-380 °C. The solid residue was of Fe_2O_3 [35].

The antibacterial assay was carried out by the agar plate diffusion method. The absorbance was measured at 520 nm.The minimum inhibitory concentration was determined by liquid dilution method [36].The solutions of ligand and complexes with 2 µg/ml,2.5 µg/ml and 3 µg/ml concentrations were prepared.The solutions of standard drug ampicilin and metal salt were also prepared in the same concentration. Inoculums of the overnight culture were prepared.0.2 ml of the inoculums was added to the test tubes containing the solutions of the compounds of different concentrations. Sterile water was added and these were incubated for 24 hours and observed for turbidity. The absorbance of the turbid solutions was measured at 520 nm.The same procedure was carried out for standard [37].High absorbance was observed at 2µg/ml concentration,less absorbance at 2.5 µg/ml and no absorbance at 3µg/ml.The metal salt solutions showed less absorbance ie better inhibitory activity than ligands and complexes.The minimum inhibitory concentration found 2.5µg/ml. Thus coordination of metal ion to ligandie formation of complexes enhances microbial activity.The metal salt solution showed better inhibitory activity.The free metal ion was found

more effective than ligand and complexes. Complexes are found more effective than the free ligands.The variation in the activity of different complexes against different organisms depends either on the impermeability of cells of the microbes or differences in the ribosomes of microbial cells [38].Chelation theory explains higher antibacterial activity of complexes. [39]. Metal chelates possess both polar and nonpolar properties.This makes them suitable for permeation into cells and tissues. The polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital upon chelation, and partial sharing of the positive charge of the metal ion with donor groups.Chelation increases the delocalization of π-electrons over the entire chelate ring and enhances the penetration of the complexes into lipid membranes [40,41] .It also increases the hydrophilic and lipophilic nature of the central metal ion, probably leading to lipo-solubility and permeability through the lipid layer of cell membranes. Lipophilicity,which controls the rate of entry of molecules into the cell, is modified by coordination, so the metal complex can become more active than the free ligand [42].

Conclusion:

Fe (III) complexes are found octahedral in nature. Magnetic study and spectral data confirmed the octahedral geometries of complexes. Ligands and the synthesized complexes were tested for antimicrobial assay.The antimicrobial activity of metal complexes is depended more on the metal center itself than on the geometry around the metal ion.Ligands exhibited less microbial activity than their metal complexes. Metal complexes are found thermally stable upto certain temperatue.

Acknolodgement :

We are grateful to Principal, Head Department of Chemistry, Pratap College Amalner for encouragement.

REFERENCES

1.Chohan Z H Sheazi S K A *Synth.React.Inorg.Met-Org.Chem.vol*.29 pp **1999**-105.
2.Jeeworth T Wah HL L K Bhowon M G Ghoorhoo D Babooram K *Synth.React.Inorg.Met-Org Chem.vol* 30 pp **2000** 1023.
3.Tossadis I A Bolos C A Aslanidis P N Katsoulos G A *Inorganica Chimica Acta vol* pp-133 **1987** 275.
4.Aggarwal R C Singh N K Singh R P *Inorg.Chim.Acta.vol*.20 pp **1981** 2794.
5.Colins C H ,Lyne P.M,*Microhiul Methods,University Park press,Baltimore* 422 **1970.**
6.Savanimi L,Chiasserini A,Gaeta,Pellerano C *Bioorg.Med Chem* 10,**2002** 2193.
7.Anten J.A.Nicholis D.Markpoulou J.M.Markopoulou *Polyhedron* 6 **1987** 1074.
8.Annelkovic K,Saldic D,Bacchi A Pelizzi G,Filipovic N,Rajkovic M *Trans.Met.Chem* 30 **2005** 243.
9.Haack T;Fattori R:Napoletano M:Pellacini F;Fronza G;Raffaini G;Ganazzoli F *Bioorg.Med.Chem*.vol.13,**2005** pp-4425.
10.Strappaghetti G;Brodi C;Giannaccini G;Betti L,*Bioorg.Med.Chem.Lett.*vol.16 **2006** pp-2575.
11.Jain R.P.Vederas J.C.*Bioorg.Med.Chem*.Lett.vol.14 **2004** pp-3655.
12.Chohan ZH, Supuran CT. J Enz Inhib Med Chem **2005**; 20(5)463
13.Jayabalakrishnan C, Natarajan K. Synth React Inorg Met -Org Chem **2001**; 31(6)983
14Chohan ZH, Humayun P, Abdul Rauf, Khalid MK, Supuran CT. J Enz Inhib Med Chem **2006**; 21(2)193
15.Jeeworth T, Wah HLK, Bhowon MG, Ghoorhoo D, Babooram K. Synth Read Inorg Met.-Org. Chem 2000; 30(6)1023
16.Dharmaraj N, Viswanalhamurthi P, Natarajan K. Transition Met Chem **2001**; 26: 105
17.Colins CH, Lyne PM. In *Microhiul Melfwds*. University Park Press **1970**; Baltimore, 422
18.Ochiai Ei-ichiro. Bioinorganic chemistry. Allyn and Bacon, Boston **1977**
19.Chohan ZH, Iqbal HS, Scozzafava A, Supuran CT, Iqbal MS *Transition met.Chem*.**2002**; 17(2,1), 87-9(15)
20.Albertini R, Pinelli S, Lunghi P. Inorg Chim Acta **1999**; 286: 134
21.Elo H, Lumme P. Inorg Chim Acta **1987**; 136(3)149
22Elo H, Lumme P. Inorg Chim Acta **1987**; 36(1)61
23.Ali MA, Kabir MH, Nazimuddin M, Majumder SMH, Tarafder MTH, Akhair M. Ind J Chem **1988**; 27A: 1064
24.Agarwal RK, Singh L, Sharma DK, Singh R. Turk J Chem **2005**; 29: 309
25.Spange, S., Vilsmeier, E., Adolph, S. and Fährmann, A. 12**, 1999** 547.
26.Tumer, M., Koksal, H., Serin, S. and Digrak, M. , 24, **1999** 13.
27.Rehman, W., Badshah, A., Khan, S. and Tuyet, L.T.A. (2009)
28.Rehman, W., Baloch, M.K. and Badshah, A. 43, **2008**, 2380
29.Rosu, T., Gulea, A., Nicolae, A. and Georgescu, R. 12,2007 782
30.Che, C. and Huang, J.S. 242, **2003** 97
31. Geary W.J., *Coord. Chem. Rev*, **1971**, 7, 81.
32. Suzuki M., Kanatomi H., Koyama H., Murase I., *Bull-Chem. Soc*, **1980**, 53, 1961.
33.Vallance R.H., Twiss D.F., Russell A.R., *A text book of Inorg. Chem, 1st Edn.* **1931**, 383.
34.Garg B.S., Kurup M.R.P., Jain S.K., Bhoon Y.K., *Transition Met. Chem*, **1991**, 16, 111.
35.Sekerci M., Yakuphanoglu F., *J. Therm. Anal. Cal***, 2004**, 75, 189.
36.SalmonS.A,WattsJ.L,Cheryal A.J.Clin.Microbial,**1995**,2435.
37.Shankar K.,Muralidhar Reddy P,RohiniR,VaddeRavindra*DerPharmacia Letter*;1,**2009**,97
38.Mounika, K., Anupama, B., Pragathi, J. and Gyanakumari, C. 2,**2010,** 513
39..Joseyphus, R.S. and Nair, M.S. 36,**2008**, 93
40.Joseyphus, R.S. and Nair, M.S. 36,**2008**, 93
41.Thangadurai, T.D. and Natarajan, K. 26,**2001**, 500
42.Farrell, N. 252,**2007,** 1