

Novel Synthesis, Spectral, Thermal and Antimicrobial Assay of Six Co-Ordinate Complexes of Fe (III) Derived from Thiosemicarbazone Moiety

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ABSTRACT

Fe (III) complexes have been synthesized by the reaction of iron (III) chloride with 5-chloro-2-hydroxyacetophenonethiosemicarbazone in presence of heterocyclic base like pyridine (py), $\alpha/\beta/\gamma$ -picoline. Thiosemicarbazone has been characterized by ^{13}C , ^1H NMR, ESI-MS as well as IR, electronic spectra. Spectral and magnetic measurements indicated Octahedral geometry for the six coordinate complexes. The thiosemicarbazone and its Fe (III) complexes have been found antifungal, antibacterial and show growth inhibitory activity against *Citrobacter*, *Acinetobacter*, *Aspergillus Niger* and *Candida Albicans*.

Keywords : Thiosemicarbazone, Metal Salts, Bioactive Metal Complexes, Antimicrobial Activity.

I. INTRODUCTION

Iron is essential to both plants and animals. It plays vital biological role in both plants and animals. Iron plays important role by providing means for transport and storage of oxygen in circulatory system. The enzymes and proteins which catalyze redox reactions contain iron [1, 2]. Iron occurs in +2 and +3 oxidation states in many compounds. Fe (II) complexes have octahedral square planer, tetrahedral or trigonal bipyramidal geometry. Fe (III) complexes are high spin or low spin and have octahedral geometry. Many iron complexes have seven coordinate pentagonal bipyramidal, octahedral geometry. Fe (III) complexes of 2 acetyl pyridine thiosemicarbazone, 2-dipyridyl ketone thiosemicarbazone, 2-formylpyridine thiosemicarbazone and substituted 2-acetylpyridine thiosemicarbazone have been reported [3-6]. Thiosemicarbazides and thiosemicarbazones are ligands which coordinate through the sulfur and one of the hydrazinic nitrogen atoms [7]. Coordination through hydrazine nitrogen atom results in a five-membered chelate ring. The thiosemicarbazones

without substituents to the thione sulfur coordinates as either a neutral or anionic NS bidentate ligand. This depends on the method of complex preparation [9], a third coordinating atom gives ONS (2-hydroxybenzaldehydethiosemicarbazones) [10] or NNS (2-acetylpyridine thiosemicarbazones) [8] donor tridentate ligands. Higher denticity involving one or more thiosemicarbazone, as well as monodentate coordination have been reported [8]. Thiosemicarbazones show antimalarial activities. Thiosemicarbazones obtained from 2-acetylpyridine was reported by Klaymaner al. [11] for antimalarial activity against *Plasmodium berghei* in mice. The molecular features essential for activity were 2-pyridylethylidene, thiocarbonyl sulfur, and bulky or cyclic substituents at the terminal N(4)-atom. The most active 2-acetylpyridine thiosemicarbazones were N(4)-phenyl and those with azacyclic substituents.

In the present work the synthesis, spectral characterisation and biological studies of six coordinate complexes of Fe (III) with 5-chloro 2-

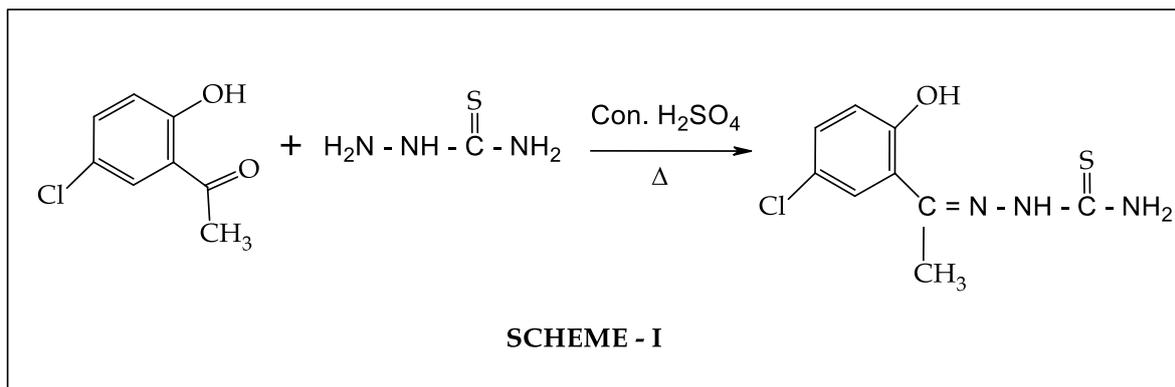
hydroxy acetophenonethiosemicarbazone have been reported.

II. EXPERIMENTAL

Materials and instrumentation

The thiosemicarbazone was synthesized by refluxing 5-chloro 2-hydroxy acetophenone and thiosemicarbazide in ethanol in the mole ratio 1:1 for 3 hours. The pale yellow product obtained was

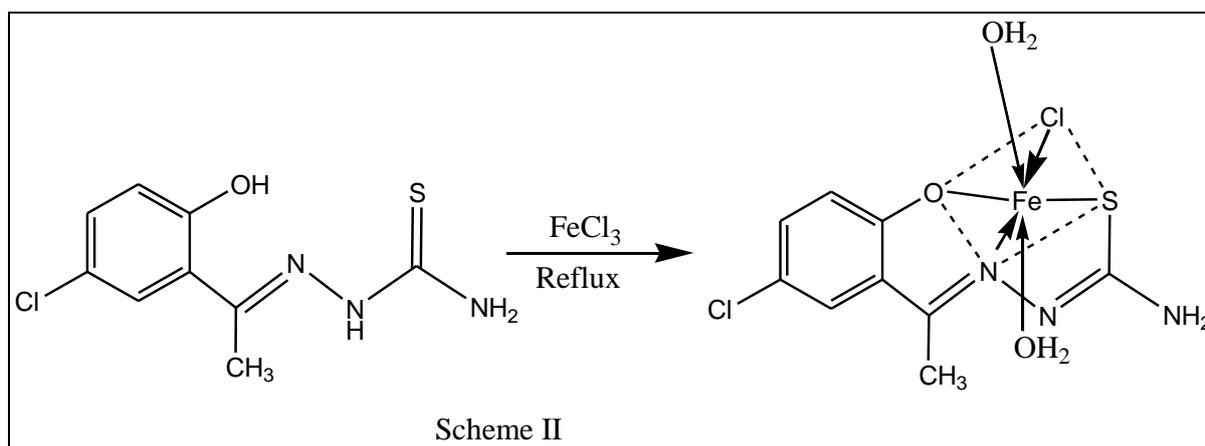
filtered and washed with cold ethanol and then diethyl ether. It was recrystallised by hot ethanol and dried over P₂O₅ in vacuum.



Preparation of complex

FeCl₃ salt was dissolved in absolute ethanol. The ethanolic solution of thiosemicarbazone in slight excess over the metal:ligand ratio of 1:1 was added to it dropwise and with constant

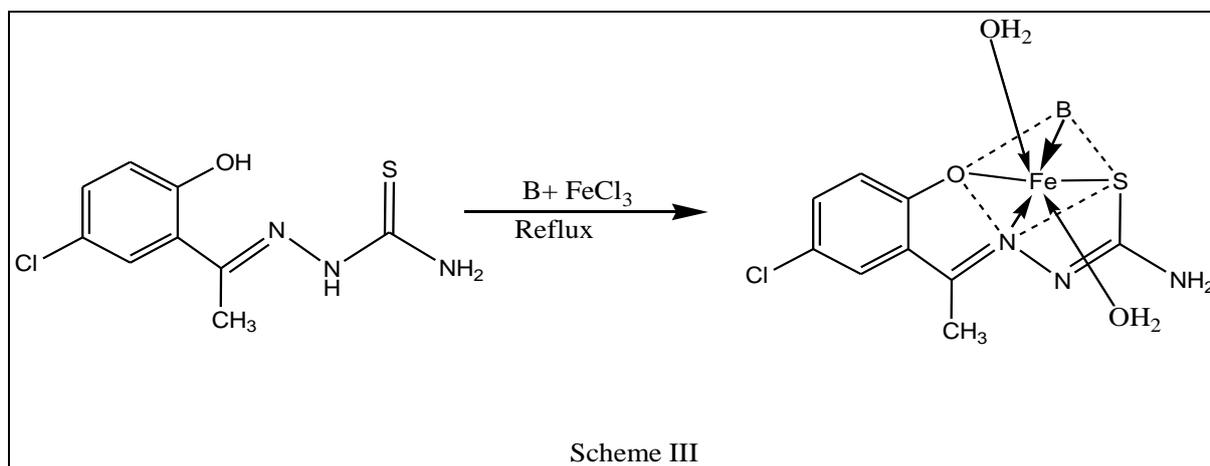
stirring. The reaction mixture was refluxed for three hours. The dark brown product thus obtained was filtered and washed well with absolute ethanol and then diethyl ether and dried over P₂O₅ in vacuum.



Preparation of complexes with heterocyclic bases

The complex Fe.L.B (B is heterocyclic base like pyridine, α -picoline, β -picoline, γ -picoline) was synthesized by adding slowly ethanolic solution of FeCl₃, heterocyclic base to the hot ethanolic solution of ligand in the ratio 1:1:1 and refluxing reaction

mixture for three hours. The brown adduct obtained was filtered and washed with hot water, cold ethanol and diethyl ether and dried over P₂O₅ in vacuum.



(B= pyridine, α -picoline, β -picoline, γ -picoline)

Physical measurements-

Magnetic susceptibility measurements were measured by Faraday method. IR spectra were recorded in the range 4000-200 cm^{-1} range. 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. Conductivity measurements were carried out on conductivity Bridge, Systonics conductivity meter-

304. Thermo gravimetric analysis was carried out in the temp rnge 30-800oC. UV-Visible spectra were measured on Jasco UV-visible double beam spectrophotometer. Metal in the complex and adducts was estimated by standerdizedxylenol orange as an indicator. Chloride in the complex was estimated by Mohr's method.

Table 1. Physical properties:

Compounds	Colour	Empirical Formula	Molar conductance $\text{Ohm}^{-1}\text{cm}^2\text{mole}^{-1}$	Magnetic Moment B.M.
L	Yellow	$\text{C}_9\text{H}_{10}\text{ClN}_3\text{OS}$	-	-
$\text{Fe-L.Cl.}(\text{H}_2\text{O})_2$	Brown	$\text{C}_9\text{H}_{12}\text{N}_3\text{O}_3\text{Cl}_2\text{SFe}$	40.2	5.22
$\text{Fe.L.Py.}(\text{H}_2\text{O})_2$	Brown	$\text{C}_{14}\text{H}_{17}\text{N}_4\text{O}_3\text{ClSFe}$	38.6	5.48
$\text{Fe.}\alpha\text{-Pico.}(\text{H}_2\text{O})_2$	Brown	$\text{C}_{15}\text{H}_{19}\text{N}_4\text{O}_3\text{ClSFe}$	35.7	5.54
$\text{Fe.L.}\beta\text{-Pico.}(\text{H}_2\text{O})_2$	Brown	$\text{C}_{15}\text{H}_{19}\text{N}_4\text{O}_3\text{ClSFe}$	40.4	5.47
$\text{Fe.L.}\gamma\text{-Pico.}(\text{H}_2\text{O})_2$	Brown	$\text{C}_{15}\text{H}_{19}\text{N}_4\text{O}_3\text{ClSFe}$	33.3	5.38

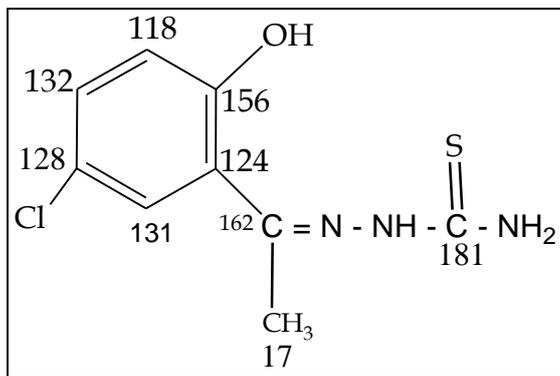
$^1\text{H-NMR}$

NMR signals at 12.00, 2.30 ppm are assigned to - OH, - CH_3 protons respectively.

No peak is observed corresponding to S-H proton, indicating it exists in thioketo form. ^4NH shows little low field position(8.50 ppm) could be due to the deshielding caused by - $\text{N} = \text{C}$ of the system

$\text{N} = \text{CSH} = \text{NH}$. Signal at 11.7 ppm is due to ^2NH . Aromatic protons show multiplets at 6.7, 7.20 and 7.63 ppm.

$^{13}\text{C-NMR}$ (DMSO-D_6): δppm 118 (C = C), 132 (C = C), 128 (C = C - Cl), 131 (C = C), 124 (C = C), 156 (C = C - OH), 162 (C = N), 181 (C=S), 17 (C- CH_3).



(Calcd) found ESI-MS m/z , ion M^+ : $C_9H_{10}ClN_3OS$ (243.70) 243.20, $C_9H_{12}N_3O_3Cl_2SFe$ (369.16) 369.60, $C_{14}H_{17}N_4O_3ClSFe$ (412.81) 412.09, $C_{15}H_{19}N_4O_3ClSFe$ (426.84) 426.14, $C_{15}H_{19}N_4O_3ClSFe$ (426.84) 426.54, $C_{15}H_{19}N_4O_3ClSFe$ (426.84) 426.91.

Analytical data

1.L: % C 44.07 (44.55), % H 4.81 (4.10), % N 17.92 (17.34), % S 13.91 (13.26)

2.Fe-L.Cl.(H₂O)₂: % Fe 15.12 (15.70), %Cl 9.07 (9.60), %C 29.96 (29.28), %H 3.77

(3.28), %N 11.93 (11.38), %S 8.08 (8.68).

3. Fe.L.Py.(H₂O)₂ :% Fe 13.14 (13.57), %C 40.03 (40.73), %H 4.74 (4.15), %N 13.04 (13.57), %S 7.11 (7.77).

4.Fe.α-Pico.(H₂O)₂: % Fe 13.80 (13.12), %C 42.92 (42.21), %H 4.03 (4.49), %N 13.78 (13.13), %S 7.07 (7.51).

5.Zn.L β-Pico.(H₂O)₂: % Zn 13.82 (13.12), %C 42.84 (42.21), %H 4.10 (4.49), %N 13.84 (13.13), %S 7.12 (7.51).

6.Zn.L.γ-Pico.(H₂O)₂: % Zn 13.80 (13.12), %C 42.72 (42.21), %H 4.80 (4.49), %N 13.72 (13.13), %S 7.19 (7.51).

Table 2. Electronic spectral data (cm⁻¹)

Compound	Mode	MLCT	n→π*	π→π*
L	DMF	-	30965	35850
Fe-L.Cl.(H ₂ O) ₂	DMF	25600	32440	34880
Fe.L.Py.(H ₂ O) ₂	DMF	25644	32477	34850
Fe.α-Pico.(H ₂ O) ₂	DMF	25650	32495	34800
Fe.L β-Pico.(H ₂ O) ₂	DMF	25686	32455	34862
Fe.L.γ-Pico.(H ₂ O) ₂	DMF	25625	32465	34888

Infrared Spectroscopic data (cm⁻¹)

1.L:ν (- OH) 3310; ν (C = N) 1630; ν (- C = S) 788, 1365; ν (N - N) 1058; ν (N-H) 3235; ν (C - O) 1287.

2 [Fe-L.Cl.(H₂O)₂]: ν (C = N) 1612; ν (C = N-N=C) 1545, ν (C-S) 710, 1315, ν (N-N) 1120, ν (M - N) 440, ν (M-O) 515, ν (M-S) 315, ν (C - O) 1220, ν (Fe-Cl) 380. ν (H₂O) 3500, 3540.

3.[Fe.L.Py.(H₂O)₂]: ν (C = N) 1580; ν (C = N-N=C) 1540, ν (C-S) 714, 1320; ν (N-N) 1125, ν (M - N) Base 265, ν (M - N) 445, ν (M - O) 520, ν (M-S) 320, ν (C - O) 1225, Band due to HB 1460, ν (H₂O) 3540, 3565.

4.[Fe.α-Pico.(H₂O)₂]: ν (C = N) 1585; ν (C = N-N=C) 1544, ν (C-S) 717, 1325, ν (N-N) 1130, ν (M - N) Base 270, ν (M - N) 450, ν (M - O) 525, ν (M-S) 325, ν (C - O) 1230, Band due to HB 1460, ν (H₂O) 3545, 3570.

5.[Fe.L β-Pico.(H₂O)₂]: ν (C = N) 1588; ν (C = N-N=C) 1550, ν (C-S) 720, 1330, ν (N-N) 1135, ν (M - N) Base 275, ν (M - N) 455, ν (M - O) 530, ν (M-S) 328, ν (C - O) 1235, Band due to HB 1470, ν (H₂O) 3550, 3575.

6.[Fe.L.γ-Pico.(H₂O)₂]: ν (C = N) 1592; ν (C = N-N=C) 1555, ν (C-S) 725, 1335, ν (N-N) 1140, ν (M - N) Base 280, ν (M - N) 460, ν (M - O) 535, ν (M-S) 330, ν (C - O) 1240, Bands due to HB 1475, ν (H₂O) 3555, 3580.

TGA analysis data:

The TGA curves of complexes were recorded between the temperatures 30 °C to 800 °C

- 1.[Fe-L.Cl.(H₂O)₂]: First step, 115.0 °C, Mass loss 4.90 % second step, 130.0 °C, Mass loss, 9.90 % Third Step 245.0 °C, Mass loss, 30.0 % Fourth Step, 370.0 °C, Mass loss 60.0 %, Residue 800 °C, % of Fe₂O₃, 70.92 (70.43).
- 2.[Fe.L.Py.(H₂O)₂]:First step, 115 °C, Mass loss 4.28 % second step,131.40 °C, Mass loss, 8.55 % Third Step 250 °C, Mass loss, 32.02 % Fourth Step, 369.30°C, Mass loss, 60.01 %, Residue, 792.34 °C, % of Fe₂O₃, 62.14 (62.98).
- 3.[Fe.α-Pico.(H₂O)₂]:First step, 115.35 °C, Mass loss 4.15 % second step, 130.50 °C, Mass loss, 8.26 % Third Step 249.40 °C, Mass loss, 30.47 %
- 4.[Fe.L β-Pico.(H₂O)₂]:First step, 115.26 °C, Mass loss 4.17 % second step, 132.14 °C, Mass loss, 8.27 % Third Step 250 °C, Mass loss, 29.02 % Fourth Step, 366.35°C, Mass loss, 65.27 %, Residue, 779.50 °C, % of Fe₂O₃, 60.33 (60.91).
- 5.[Fe.L.γ-Pico.(H₂O)₂]:First step, 114.92 °C, Mass loss 4.16 % second step, 131.20 °C, Mass loss, 8.25 % Third Step 245.43 °C, Mass loss, 33.28 % Fourth Step, 370.28 °C, Mass loss 60.78 %, Residue 800 °C, % of Fe₂O₃, 60.27 (60.91).

Antimicrobial Assay(Agar well diffusion method)

Table.3 % Activity index of L , Fe (III) complexes and standered

Compound % Activity Index	Citrobactor		Acinetobactor		Asper gillusNigar		Candida Albicans	
	10 ⁻³ M	10 ⁻⁴ M	10 ⁻³ M	10 ⁻⁴ M	10 ⁻³ M	10 ⁻⁴ M	10 ⁻³ M	10 ⁻⁴ M
L	25.10	22.30	40.10	35.30	54.50	40.30	63.60	51.02
Fe-L.Cl.(H ₂ O) ₂	44.12	35.18	50.10	35.66	69.30	65.20	81.30	65.15
Fe.L.Py.(H ₂ O) ₂	49.35	42.40	51.12	37.72	70.25	66.30	82.32	67.10
Fe.α-Pico.(H ₂ O) ₂	47.10	43.60	50.50	35.45	69.20	65.70	79.15	61.15
Fe.Lβ-ico.(H ₂ O) ₂	37.25	33.30	45.20	32.40	62.65	53.95	65.40	52.30
Fe.L.γ-co.(H ₂ O) ₂	35.30	32.35	41.25	30.45	58.70	51.90	60.45	50.02
Standered	100	100	100	100	100	100	100	100
FeCl ₃	112.10	102.25	103.38	100.30	114.20	108.10	116.30	112.20

(Standered-Cefodoxine,Fluconazole)

% activity index was calculated by the formula

$$\% \text{ Activity Index} = \frac{\text{Zone of inhibition of test compound}}{\text{Zone of inhibition of standard(diameter)}} \times 100$$

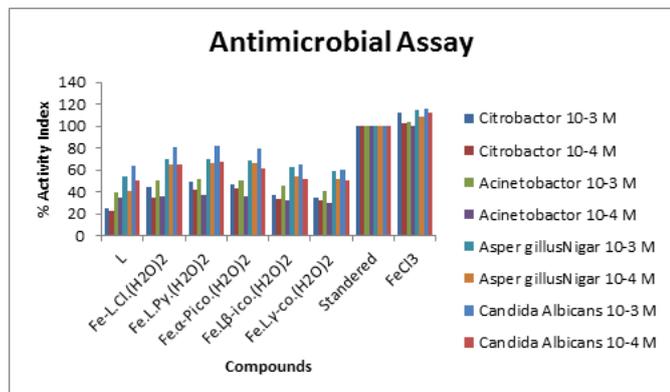


Figure 1. Antimicrobial Assay

III. RESULTS AND DISCUSSION

Physical data of thiosemicarbazone and its complexes are presented in Table 1. Elemental analysis data confirmed with 1:1 ratio of metal ion, thiosemicarbazone for complex and 1:1:1 ratio for metal, thiosemicarbazone and heterocyclic base for all adducts. The complex and all adducts are soluble in DMF in which conductivity measurements were made (30°C), showing all complexes to be non electrolyte [12]. The magnetic moments were measured at room temperature (30°C) in polycrystalline state showed magnetism between 5.22-5.54. Mass spectral data confirmed the structure of the thiosemicarbazone as indicated by molecular ion peak ($M + 1$) corresponding to their molecular weights. UV-visible spectra of metal complexes in DMF solution are presented in Table no.2 The thiosemicarbazone has band $\pi - \pi^*$ band [13-15] at 35,850 cm^{-1} and $n - \pi^*$ [16,17] at 30,965 cm^{-1} . No absorption bands observed above 500 nm in DMF solution indicating the absence of d-d transition bands. These bands are slightly shifted to higher energy side in complexes. The $\pi - \pi^*$ absorption bands are shifted to longer wavelength in complexes due to the weakening of the C=S bond conjugation system gets enhanced on complexation [18,19]. The reduction in intensity of $n - \pi^*$ bands in the complexes is due to transfer of lone pair of electrons to the metal and coordination of azomethine nitrogen. MLCT bands are broad in the complexes and observed in the

region 25000-26000 cm^{-1} which are assignable to Fe→S transitions.

There is no band in the region 2600-2800 cm^{-1} region of the IR spectrum of thiosemicarbazone complex showed the absence of thiol tautomer in the solid state. Coordination through azomethine nitrogen $^7\text{C}=\text{N}^1$ shifts the frequency to the lower side [20] A new band in the range 440-460 cm^{-1} confirms the coordination of azomethine nitrogen [21,22]. The increase in frequency of $^1\text{N}-^2\text{N}$ may be due to the increase in double bond character off-sets the loss of electron density via donation to the metal. This confirms the coordination of thiosemicarbazone through the azomethine nitrogen atom. The band $^2\text{N}-\text{H}$ of thiosemicarbazone disappears in the complexes indicating the deprotonation of the $^2\text{N}-\text{H}$ proton. A thioamide band which is partly due to C=S found at 1365 and 788 cm^{-1} in thiosemicarbazone shifted to lower side in complexes indicating coordination through thiolate sulfur [23]. New bands in 315-330 range due to Zn-S confirms involvement of sulfur coordination. The phenolic oxygen indicates the third coordination on loss of OH protons. This causes shifting of $\nu(\text{C}-\text{O})$ 1287 cm^{-1} in uncomplexed thiosemicarbazone to lower side in the complexes. New bands due to Fe-O in complexes in the range 515-535 cm^{-1} confirm coordination through oxygen. The heterocyclic base nitrogen atom occupies fourth coordination site. The band is assigned for $\nu(\text{Fe}-\text{N})$ due to heterocyclic base in 265-280 cm^{-1} range in the spectra of all complexes. The characteristic bands due to coordinated heterocyclic bases are also observed in IR spectra of all complexes [24-26].

The TGA curves of Fe (III) complex and adducts were recorded within a temperature range 30-800°C. The decomposition of complex proceeded in steps. Hydrated molecules of water were lost in between 30-110°C. Then two coordinated water molecules were lost in the temperature range 114-116°C. Mass lost at this stage is about 4-5 %. No change observed ~ 130°C after that break observed in the curves due to evaporation of molecule of thiosemicarbazone. The

remaining part of thiosemicarbazone removed from the coordination sphere at $\sim 600^{\circ}\text{C}$. Finally the metal oxides were formed above 600°C . The decomposition was complete at $\sim 800^{\circ}\text{C}$.

It has been found that Fe (III) complex and adducts were thermally stable up to 114°C and decomposition started at this temperature. The second step temperature is in the range of $130\text{--}132^{\circ}\text{C}$. The third step temperature is in the range of $240\text{--}250^{\circ}\text{C}$. The solid residue is Fe_2O_3 . The complexes prepared with different metals decompose in steps. It is concluded that the thermal stability of the complex is due to the coordination of metal ion to thiosemicarbazone [27].

The antibacterial assay was determined using the agar well diffusion method. The activity was determined by measuring the diameter of the inhibition zone (in mm). Biological activity was measured in two different molar concentrations (10^{-3}M , 10^{-4}M). The chelate $\text{Fe.L.py}(\text{H}_2\text{O})_2$ showed maximum activity against bacterial and fungal species than free ligand. The results of antibacterial and antifungal studies are given in Table 3. The thiosemicarbazone was found less active than its iron complexes. This might be due to chelation which reduces the polarity of the central metal atom because of the partial sharing of its positive charge with donor groups and possible π -electron delocalisation within the whole chelating ring. So the lipophilic nature of the central metal atom increases, which favours the permeation of the solution of complexes through the lipid layer of the cell membrane [28]. *Acinetobacter* and *Citrobacter* are gram -ve bacterial species. In gram negative bacteria the outer membrane is thin and they possess outer membrane. So, it is not easy for the complexes to diffuse inside the bacterial cell. Good inhibitory activity was found in case of yeast *Candida albicans* [29]. The metal chloride salt was more effective than complexes. This shows that free metal ions are more effective than one bound in complexes. But it is toxic to treat bacterial diseases by using free metal ions. So it can be used in coordinated form with

thiosemicarbazone. The general sequence of % activity index can be represented as -

Standard > metal salt > complexes > ligand.

The % activity index decreases on dilution i.e. it is more in concentrated solution (10^{-3}).

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