Antimicrobial screening and thermal stability study of six co-ordinate complexes of Co (II) derived from hydrazide ligands

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Abstract :- The reaction of acetohydrazide with 3,5-dichloro-2-hydroxy acetophenone, 5-chloro-2-hydroxy acetophenone and 4,5-dichloro-2-hydroxy acetophenone was carried out to prepare hydrazide ligands..Complexes with Co (II) were synthesized by reaction of $CoCl_2.6H_2O$ with hydrazide lignd 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 13CNMR) magnetic measurement and conductivity at different temperature.The metal complexes and corresponding ligands were screened against bacterial parasites. It has been observed that the Co (II) complexes were found more biologically active than corresponding hydrazide ligands.
Keywords: 1:2 ratio,bioactive metal complexes,paramagnetism,$CoCl_2.6H_2O$.

INTRODUCTION

The complexes with hydrazides   possess biological properties such as antibacterial [1-3],antifungal [4],anti-tumor [5-8],anti-malarial [9],anti-cancer [10],anti-inflammatory [11]. Metal complexes of Schiff base ligands possess wide range of applications in the biological, analytical, clinical, and industrial areas [12]. The transition metal complexes of Schiff base ligands are important , not only due to their spectroscopic properties and applications [13] but also due to their antifungal, antibacterial and antitumor activities [14]. 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 [15-18]. The Co (III) complexes $[Co(ONSH)_2]Cl$ and $[Co(ONSH)(ONS)].H_2O$ have been studied[19].

In this research work the synthesis, stability study,spectral characterization,conductivity measurement at different temperature and biological screening of six coordinate complexes of Co (II) with hydrazide ligands have been reported.

II MATERIALS AND METHODS

 The chemicals used are of A.R.grade. Magnetic susceptibility measurement was carried out by Faraday method at room temperature. Infra red spectra were recorded in solid state in the range 4000-200 cm^{-1} range.Refluctance spectra was recorded in solid state.Thermogravimetric anaysis was carried out in the temperature range 30-800ºC.Metal was estimated using standardized E.D.T.A , xylenol orange as an indicator and hexamine as buffer .

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.
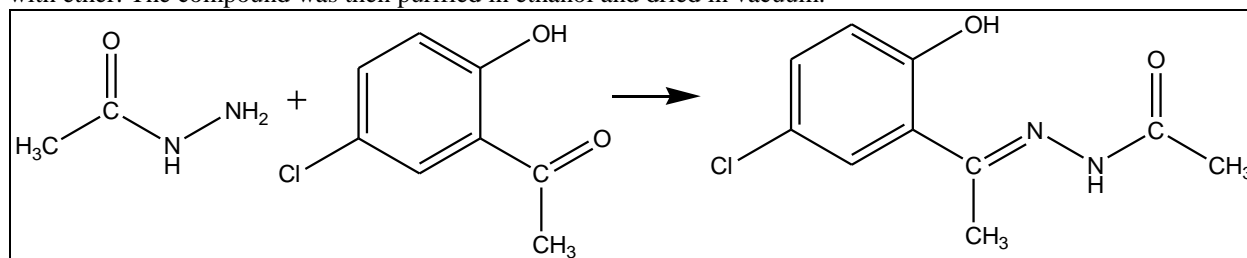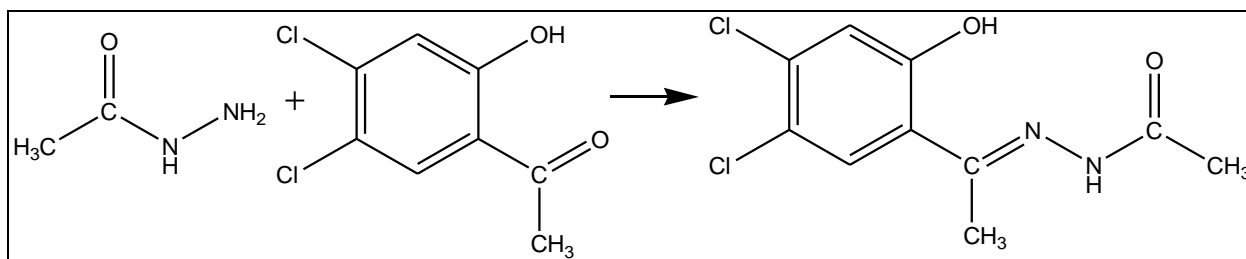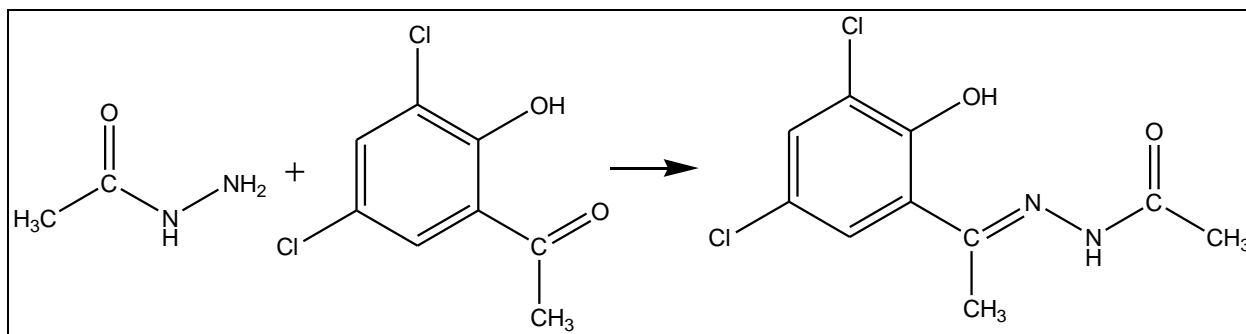
Synthesis of complex

The complexes were synthesized by adding slowly ethanolic solution of $CoCl_2.6H_2O$ (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	$C_{10}H_{10}O_2N_2Cl_2$		
Co-L$_2$	Brown	$C_{20}H_{24}N_4O_6Cl_2Co$	41.8	4.52
Co-L$_2$'	Brown	$C_{20}H_{22}N_4O_6Cl_4Co$	55.5	4.60
Co-L$_2$''	Brown	$C_{20}H_{22}N_4O_6Cl_4Co$	58.7	4.58

Conductivity measurement

The complexes are soluble in DMF so conductivity measurement measured using DMF solutions and equivalent conductance was calculated. The 0.001 M solution of complexes was prepared in different percentages of DMF-ethanol mixture and the parameter of solution under study was calculated at temperature 300 K, 305 K and 310 K.

Equivalent conductance at 300 K			
DMF-Ethanol mixture	Co.L$_2$.	Co.L$_2$'	Co.L$_2$''
75%	33.0	35.7	37.5
80%	37.4	40.8	39.7
85%	45.3	42.0	41.6
90%	47.3	48.0	45.3
95%	50.5	50.3	47.7
100%	53.7	52.8	50.5
Equivalent conductance at 305 K			

DMF-Ethanol mixture	Co.L$_2$.	Co.L$_2$'	Co.L$_2$''
75%	40.7	40.5	41.1
80%	42.5	41.2	43.4
85%	45.3	45.2	45.4
90%	52.5	50.3	47.2
95%	55.3	52.4	49.7
100%	58.5	56.3	52.4

| Equivalent conductance at 310 K			
DMF-Ethanol mixture	Co.L$_2$.	Co.L$_2$'	Co.L$_2$''
75%	43.7	41.2	41.1
80%	45.8	43.2	44.5
85%	47.6	45.5	50.4
90%	51.4	52.3	52.2
95%	56.2	53.4	54.7
100%	62.5	57.5	60.1

^{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.
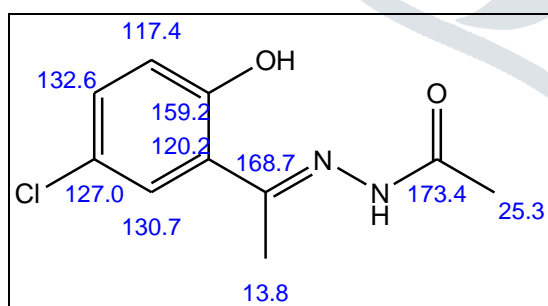
^{13}C-NMR (L) δppm　　　　　　　　　　**^{13}C-NMR (L') δppm**
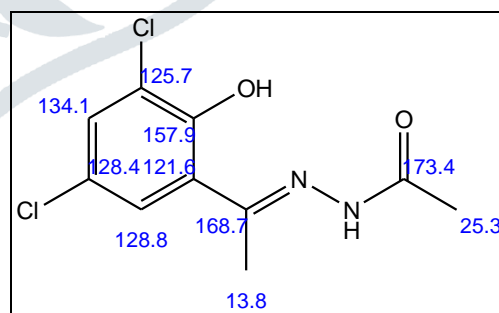
^{13}C-NMR (L'') δppm
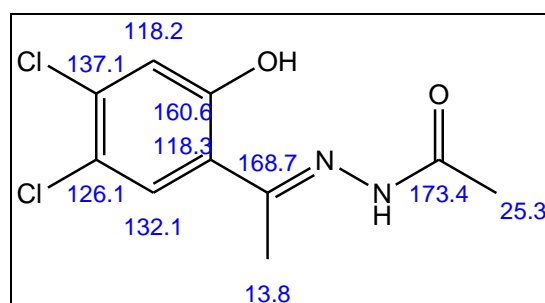
ESI-MS m/z, ion M⁺(Calcd) found

$C_{10}H_{11}N_2O_2SCl$ (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, $C_{20}H_{24}N_4O_6Cl_2Co$ (546.25) 546.91, $C_{20}H_{22}N_4O_6Cl_4Co$ (615.13) 613.81, $C_{20}H_{22}N_4O_6Cl_4Co$ (615.13) 615.75.

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)
Co.L₂	10.07 (10.95)	43.12 (43.97)	4.81 (4.43)	10.82 (10.26)	17.82 (17.57)
Co.L'₂	9.91 (9.58)	39.71 (39.05)	3.03 (3.60)	9.88 (9.11)	15.11 (15.61)
Co.L''₂	9.87 (9.58)	39.62 (39.05)	3.10 (3.60)	9.81 (9.11)	15.25 (15.61)

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
Co.L₂	17,615	23,720	32,250	43,200
Co.L₂'	17,675	23,680	32,560	42,350
Co.L₂''	17,760	23,900	32,450	42,900

Infrared Spectroscopic data (cm⁻¹)

IR-spectral data

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

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

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

4.Co.L₂: ν (- OH) 3220,ν (C = N) 1565,ν (N-N) 1160, ν (N-H) 3255ν (M - N) 455, ν (M - O) 540,ν (C - O) 1215

5.Co.L₂': ν (- OH) 3260,ν (C = N) 1570,ν (N-N) 1175, ν (N-H) 3269ν (M - N) 465, ν (M - O) 545,ν (C - O) 1225.

6.Co.L₂'': ν (- OH) 3280,ν (C = N) 1575,ν (N-N) 1185, ν (N-H) 3262ν (M - N) 470, ν (M - O) 550,ν (C - O) 1230.

TGA ANALYSIS DATA:

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

1.**Co.L₂**: First H₂O 110⁰C Mass loss 3.81, Second H₂O 111⁰C Mass loss 6.03 First step, 114 °C, Mass loss 4.52 % second step, 140.0 °C, Mass loss, 19.30 % Third Step 249.0 °C, Mass loss, 38.0 % Fourth Step, 371.0 °C, Mass loss .66.0 %, Residue 800 °C, % of CoO, 13.05(13.72).

2.**Co.L₂'**: First H₂O 109⁰C Mass loss 2.18, Second H₂O 111⁰C Mass loss 5.11, First step, 121 °C, Mass loss 11.23 % second step,352.0 °C, Third Step 242.0 °C, Mass loss, 42.02 % Fourth Step, 379.0 °C, Mass loss .69.0 %, Mass loss, 59.0 %, Residue, 781 °C, % of CoO, 12.77 (12.18).

3.**Co-L₂''.** : First H₂O 108⁰C Mass loss 2.25, Second H₂O 112⁰C Mass loss 5.20,First step, 123 °C, Mass loss 11.57 % second step 363 °C, Mass loss, 60.0 %, Third Step 256.0 °C, Mass loss, 46.02 % Fourth Step, 371.0 °C, Mass loss .69.0 %, Residue 788 °C, % of CoO, 12.81 (12.18).

**IX BIOLOGICAL ACTIVITY (AGAR PLATE DIFFUSION METHOD)**

Table.6 Minimum Inhibitory concentration L, Co (II) 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
Co.L₂	0.61	0.31	0.62	0.19	0.60	0.21	0.62	0.22

$Co.L_2'$	0.59	0.30	0.59	0.17	0.59	0.19	0.61	0.21
$Co.L_2''$	0.58	0.29	0.56	0.16	0.57	0.17	0.60	0.20
$CoCl_2.6H_2O$	0.43	0.20	0.21	0.11	0.46	0.15	0.36	0.14
Standard	0.16	0.13	0.16	0.10	0.15	0.11	0.16	0.11

(Std-Amphiciline)

Fig.1 % Activity Index Bar Graph
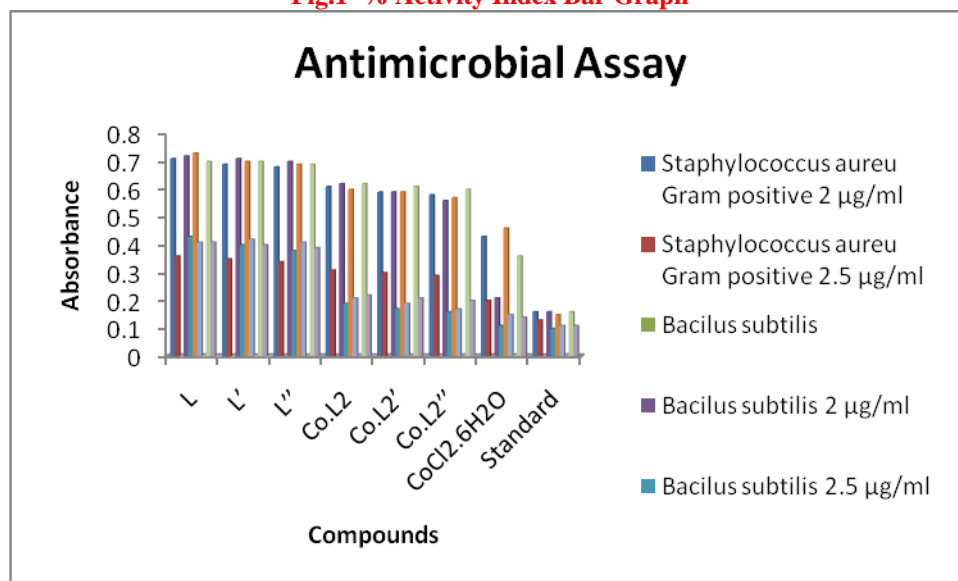
RESULTS AND DISCUSSION

 1:2 ratio of metal ion and ligand in all complexes.The complexes are insoluble in DMF solvent.They conductivity measurements were carried pot in DMF.All complexes showed electrolyte behaviour [20].Mass spectral data confirmed the structure of ligands and complexes.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 is found that equivalent conductance of an electrolyte increases with increase in dilution. In dilute solution conductance is more. Equivalent conductance increases with dilution at 300 K, 305K,310 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 $\Pi – \Pi*$ (aromatic ring) and $n – \Pi*$ transitions respectively.The broad bands in 32,000 – 33,000 range are assigned for $n – \Pi*$ transitions in all complexes [21]. The shift of $\Pi – \Pi*$ 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}. These are assigned to ${}^4T_1g\ (F)\to{}^4T_2g\ (F)$,${}^4T_1g(F)\to{}^4A_2g\ (F)$ and ${}^4T_1g(F) \to {}^4T_1g(P)$ respectively expected for d^7 system in octahedral field.The bands at 25,000 – 26,000 cm^{-1} range correspond to $L \to M$. .These bands suggested octahedral geometry around Co(II) [22].

 The coordination of azomethine nitrogen shifted $\nu(C = N)$ shifted to lower wavenumbers due to coordination of azomethine nitrogen.ν(N-N) shifted to higher wavenumbers confirms coordination of azomethine nitrogen [23].The band at 455 – 470 cm^{-1}is assignable to ν(Co– N) confirmed the coordination of azomethine nitrogen.The bands at 540–550 cm^{-1} is assignable to ν(Co-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 111°C corresponding to mass loss 2.0-6.0 %. Decomposition proceeded in 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 600°C.The decomposition was completed at ~800 °C.It has been found that Co (II) complexes are stable up to 200°C and decomposition took place above this temperature and completed in the temperature range 300-370°C. The second steps are in the range of 310-380 °C. The solid residue was of CoO [24].

The antibacterial assay was carried out by the agar plate diffusion method.Activity was measured by measuring the absorbance at 520 nm. Ligands were found less active than all complexes.The minimum inhibitory concentration was determined by liquid dilution method [25].The solutions of ligand and complexes with 2 µg/ml,2.5 µg/ml and 3 µg/ml concentrations were prepared in the solvent DMF.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 to each of the test tubes 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 [26].More absorbance was observed at 2µg/ml concentration,less absorbance at 2.5 µg/ml and no absorbance at 3µg/ml.The metal salt solution showed better inhibition than ligand and complexes.The minimum inhibitory concentration is 2.5µg/ml. Thus coordination of metal ion to ligand enhances microbial activity.The free metal ion was found more effective than ligand and complexes. In general, complexes are found more effective than the free ligands and the same was observed in this study, that the complexes are more active than the parent ligands. The effective activity is exhibited by Co (II) complexes towards bacterial species. The ligands show less activity. 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 [27]. The higher antibacterial activity of complexes can be explained by chelation theory of the ligand with metal ions [28] as 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 [29,30] .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. Further, 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 [31].

Conclusion:

Co (II) complexes are octahedral in nature. Magnetic moment studies and spectral data proved the octahedral geometries to all metal complexes. Ligands and the synthesized complexes were tested as antibacterial agents.The antimicrobial activity of metal complexes is depended more on the metal center itself than on the geometry around the metal ion.Ligands are found less active than its metal complexes.TGA data revealed that metal complexes are thermally stable.

Acknolodgement :

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