

The Influence of a New-Synthesized Complex Compounds of Ni (II), Cu (II) And Fe (II) Containing A Ligand Having Tetraoxotetrahydrazin Moity on Some Pathogenic Bacteria.

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Abstract: Some macrocyclic complex compounds of Ni (II), Cu (II) and Fe (II) containing a ligand having tetraoxotetrahydrazin moity are synthesized by template condensation of malonodihydrazide ($C_3H_8N_4O_2$) with different aldehydes. The complexes are characterized on the basis of elemental analysis, UV-visible & IR spectroscopy, magnetic moment and conductance measurement and other physical properties. The IR spectrum study of the complex compounds suggests that ligand coordinates to metal ions through the nitrogen atoms from the tetraoxotetrahydrazin moity. Antibacterial activity of the derived complex compounds, as well as already used standard compound kanamycin, was tested on fourteen pathogenic bacteria. Given results were then compared to the efficacy of the Antibacterial activity of standard compound kanamycin used for control of these pathogenic bacteria.

I. Introduction

Naturally occurring macrocyclic-ligand transition metal complexes such as complexes of the porphyrin or corrin ring systems and the industrially important metal phthalocyanine complexes have been studied for many years. More recently, a large number of other macrocyclic ligands have been synthesized and their metal complexes have been extensively studied. The present review gives an outline of the transition-metal chemistry of this latter group of cyclic ligands with an emphasis being placed on the more recent work.

The chemistry of synthetic macrocyclic ligands can be divided into two broad divisions. Firstly there are the cyclic polyethers of the 'crown' type of which is a typical example¹. Ligands of this general category have received much recent attention because of their unusual behavior towards a range of non-transition metal ions². Few studies involving transition metal ions have been reported³ and it is evident that the majority of such polyether ligands show a limited tendency to form stable complexes with these ions⁴.

The coordination chemistry of hydrazones is an intensive area of study and numerous transition metal complexes of these ligands have been investigated⁵. The development of the field of bioinorganic chemistry has increased the interest in Schiff base complexes, since it has been recognized that many of these complexes may serve as models for biologically important species⁶. Coordination compounds derived from aroylhydrazones have been reported because of their anti-tuberculosis, antimicrobial and corrosion inhibitors⁷. The chemistry and complexation properties of macrocyclic dioxotetraamines were investigated⁸. These macrocycles contain two amino nitrogens and two amides. As with cylam and cyclen, the amino nitrogens with additional coordinating groups form new hexadentate ligands. They are able to bind to metals like copper(II) and nickel(II) with simultaneous dissociation of the two amide protons, such that metal binding is highly pH-sensitive and reversible (-a very useful property for metal-sensing applications). The copper(II) complex of a functionalised trans system at neutral and basic pH, and found very different structures according to whether just one or both of the amides are deprotonated⁹.

Template Synthesis of Macrocyclic Complexes

The rich chemistry of the complexes of macrocyclic ligands continues to be a subject of growing importance, as reflected by a number of recent reviews and books¹⁰. Much of this work has been stimulated by the recognition of the high kinetic and thermodynamic stability of the complexes formed by macrocyclic ligands, the so-called "macrocyclic effect"¹¹, and also by the realization of the key importance of cyclic ligand systems in biology, for example, the protoporphyrin IX ligand of heme, or the corrin system found in the Vitamin B₁₂ coenzyme¹². Because of this work, macrocyclic ligands are discussed routinely as an integral part of any course on transition metal coordination chemistry. However, comparatively few undergraduate experiments are available to illustrate the practical side of macrocyclic synthesis, although one example, of the synthesis of a binuclear macrocycle, was reported fairly recently¹³.

Coordination Complex of Perchlorate Ion

The recent article describing the use of [Cu(tmen)(arac)]ClO₄ as a color indicator for solvent parameters¹⁴ fails to identify the potential danger associated with the preparation and handling of this salt. Most of us are aware that "organic perchlorates are self-contained explosives"¹⁵ However, many overlook the fact that a perchlorate salt of a cation, such as a complex ion that contains an organic group or other oxidizable atoms, is also an explosive (although the conditions required to initiate an explosion vary from sample to sample). For example, one sample of Co(H₂O)₃(ClO₄)₂ detonated under a slight impact while attempts to repeat the detonation with other samples were not successful¹⁶. Such compounds must be handled with great care¹⁷, if at all. The complex [Ag(INHSAL)₂(ClO₄)] and [Ag(INHHAP)₂ClO₄] have been synthesized¹⁸ by adding ligand dropwise and mixed with metal perchlorate solution in the 2:1 molar ratio, (where ligand INHSAL = 2-hydroxybenzaldehyde) 1 NHHAP=2hydroxy acetophenone. On the basis of IR spectra showed a band at (1090-620) cm⁻¹ regions these are assignable that perchlorate coordinate to the metal.¹⁹

Biological Activity of Some Important Compounds

Complex compound is very important in bioinorganic chemistry. Over the last decade or so there has been a growing awareness of the importance of wide range of metallic and non metallic elements in biological system²⁰. Some 25 elements which are currently throughout to be essential to life, ten can be classified as trace metal ions; Fe, Cu, Zn, Mn, Co, Cr, Sn, V and Ni and four as bulk metal ions; Na, K, Mg and Ca. In addition there is some tentative evidence that Cd and Pb may be required at very low levels. There is also evidence that Sn, As and Br may possibly be essential trace elements. In the following section the out line of the chemistry and biological effects of some of the essential and polluting elements is given below.

A number of metal complexes and ligand have been shown to be chemically useful in variety of areas, e.g. As antitumor agent's antiviral agents and in the treatment of illness, for example, in haemocyanins, contain Cu and bind one molecule of O₂ for every pair of copper(I) ions. Haemocyanine is found only in molluscs and arthropods. Inorganic chemistry has been interested in developing suitable copper complexes which would mimic some of properties of haemocyanin.²¹

Aim of the Present Work

Macrocyclic complex compounds are an immense field in chemistry, especially in coordination chemistry and in bio-inorganic chemistry.

In the nature a lot of macrocyclic compounds are known (e.g. haemoglobin, myoglobin, vitamin B₁₂ coenzymes where the metal atoms are Fe, Co and the basic macrocyclic unit is porphyrin but have different functional group to the macrocycles). The chemical properties of some macrocyclic compounds resemble those of antibiotics.²² For instance, macrolactones resemble the natural metabolites such as nonactin and monactin. In the recent years considerable attention have been given to the synthesis of macrocyclic complex²³. These complex compounds have been used as a model system of biologically important materials, such as porphyrin and corins. Some of the macrocyclic ligand cannot be easily prepared from the reactants²⁴. In that case the complex compounds could be synthesised by template method. The desired macrocyclic ligand can be isolated by stripping the complex compounds²⁵. Macrocyclic tetraaza complex of Ni²⁺ act as catalyst to reduce CO₂ to CO and Fe²⁺, Mn³⁺ porphyrins have been most commonly studied catalyst.²⁶

In view of the extensive use as drugs and significant pharmacological activities of macrocyclic complexes and their derivatives, it is desired to synthesize macrocyclic complexes of Ni (II), Cu (II) and Fe (II). The synthesized macrocyclic complexes and their derivatives are expected to have microbial activity.

Therefore, considering the rapid increasing importance of macrocyclic ligand and their complexes in biology and in medicine the present work is divided into two parts:

i. Firstly, synthesis of some new macrocyclic complexes by the reactions of malonodihydrazide with Ni(II), Cu(II) and Fe(II) perchlorate in the presence of formaldehyde, acetaldehyde, butyraldehyde will be characterised by elemental analysis, UV visible and IR spectral analysis, magnetic moment and conductance measurements and some other physical properties.

ii. Secondly, study of antibacterial activity of the synthesised complexes (some test organisms such as, *Salmonella-17*, *Klebsilla*, *Shigella dysenteriae*, *Shigella shiga*, *Shigella boydii*, *Shigella sonnei*, *Shigella flexneri*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella*, *Bacillus megaterium*, *Sarcina lutea*, *Staphylococcus aureus*, *Bacillus cereus*) including the investigation of minimum inhibitory concentration of the complexes.

II. Experimental

The ligand precursor, malonodihydrazide was prepared by the literature procedure²⁷. Micro analysis for carbon hydrogen and nitrogen were obtained by using Kjeldahl Method for elemental analysis. Infrared spectra (as KBr disc) were recorded using a shimadzu FTIR-8400 spectrometer from 4000-400 cm^{-1} and uv-visible spectra on shimadzu uv-160 Spectrophotometer in DMSO. Magnetic moment measurements were done on Sherwood scientific magnetic susceptibility balance. Conductivities were measured by CG-857 Scott Gerate GmbH conductivity meter with a dip type cell having platinum electrodes in DMSO. Metals were estimated complex complex metrically using EDTA and DMG after fuming the complexes with sulfuric acid²⁸. Melting points were determined on an electro thermal melting point apparatus (model no. AZ 6512).

III. Preparation Of Macrocyclic Complexes.

Preparation Of $[\text{Ni}(\text{C}_8\text{H}_{16}\text{N}_8\text{O}_4)(\text{ClO}_4)_2]$ Complex 1.

To the aqueous malonodihydrazide, $\text{C}_3\text{H}_8\text{N}_4\text{O}_2$ (0.792 g, 6 mmol in 10 mL water) formaldehyde solution (0.48 g, 6 mmol 37%) was added. To the above solution nickel(II) perchlorate hexahydrate (1.096 g, 3 mmol in 10 mL water) was added and the whole mixture was refluxed with constant stirring for two hours and cooled down. A blue precipitate was formed immediately. The product was washed with ethanol for three times and dried in a vacuum desiccator over anhydrous CaCl_2 . The melting point of the compound was 270°C and yield was 1.894 g (80%). The compound was soluble in DMSO and insoluble in acetone, ethanol, water and chloroform. Same procedure was applied for the preparation of complexes 2, and 3 using acetaldehyde and cinnamaldehyde were the reaction mixture was refluxed for 12 and 20 hours respectively. Similarly the complexes (4-6) were Synthesized by the reaction of malonodihydrazide, Copper(II) Perchlorate and Corresponding aldehydes (viz., formal dehyde acetaldehyde, cinnamaldehyde) respectively. The complexes (4-6) were prepared by refluxing for half an hour, 4, 4 hours respectively.

Similarly the complexes (7-9) were Synthesized by the reaction of malonodihydrazide, Iron(II) Perchlorate and Corresponding aldehydes (viz., formal dehyde acetaldehyde, cinnamaldehyde) respectively. The complexes (7-9) were prepared by refluxing for 2, 2 and 4 hours respectively.

IV. Result And Discussion

Macrocyclic Complexes Of M (II)

Reactions of malonodihydrazide with metal (II) perchlorate hexahydrate (where $\text{M}=\text{Ni}(\text{II}), \text{Cu}(\text{II}), \text{Fe}(\text{II})$) in presence of formaldehyde, acetaldehyde and cinnamaldehyde give some 16 member macrocyclic complex as described above Complexes (1-9) are characterized on the basis of elemental analysis, magnetic moment & conductance measurements, UV-visible spectra & infrared studies and other physical properties, like melting point, solubility, colour etc. Molar conductance data of the complexes (1-9) are shown in Table 4.3. The conductance values of the complexes suggested that they are non-electrolytic in nature²⁹.

The infrared spectra of the complexes (1-9) are shown as spectral data (Table 4.5) of the complexes showed a strong and broad band at $(3246-3265) \text{cm}^{-1}$ which is assigned for the $\nu(\text{NH})$ stretching³⁰.

Due to coordination the $\nu(\text{N-H})$ stretching of the amide group goes to the higher field at $(3246, 3265) \text{cm}^{-1}$ region as compared to the starting material malonodihydrazide³¹. In the complexes the terminal- NH_2 group of malonodihydrazide condensed with the aldehyde moiety form a new secondary¹-NH group which may appear at the same region (or overlape) as to the amide-NH group as a result the $\nu(\text{N-H})$ band appear as a strong and broad band. [The starting material malonodihydrazide have three $\nu(\text{N-H})$ bands at $(3248, 3213, 3050) \text{cm}^{-1}$. The bands at $(3248, 3050) \text{cm}^{-1}$ for the asymmetric and symmetric $\nu(\text{N-H})$ stretching of the terminal- NH_2 moiety and 3213cm^{-1} for amidic (N-H) group]. The complexes showed a broad band at $(2920-2972) \text{cm}^{-1}$ is suggested for the $\nu(\text{C-H})$ stretching of aliphatic moiety³². The complexes showed a strong band at $(1649-1674) \text{cm}^{-1}$ which represent the $\nu(\text{C=O})$ of NH-NH-CO-CH_2 moiety³². Three or four band at $(625-1145) \text{cm}^{-1}$ region also indicated the $\nu_1, \nu_2, \nu_3, \nu_4$ bands of (ClO_4^-) moiety. These stretching frequency is suggested the coordination of perchlorate to the metal through the O atom³³. A medium band at $(407-412) \text{cm}^{-1}$ region is tentatively attributed to the $\nu(\text{M-N})$ mode^{34,35}. indicating the coordination of the ligand to the metal through the nitrogen atom.

The magnetic moment measurement data (Table 4.2) of the Ni(II) complexes (1-3) showed (2.82-3.14) B.M. These values correspond to two unpaired electrons of Ni(II) d^8 system suggest the octahedral environment of the complexes which are consistent with the literature value¹. The Copper complex (4-6) showed (1.33-1.77) B.M corresponding to one unpaired electron of octahedral Cu(II) d^9 system. The Iron(II) complexes (7-9) showed (4.17-4.80) B.M corresponding to four unpaired electron of octahedral Fe(II) d^6 system. The elemental analyses (C, H and N) (Table 4.1) and metal estimation data (Table 4.3) of the complexes are consistent with the proposed formula.

The UV-visible spectra of the complexes (1-3) are shown (Table 4.4) band at 450,550 nm, (1) at 400,600 nm (2) and at 410, 650 nm, (3) represent the d-d transition of ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$, ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$, which suggested the octahedral geometry of the Ni(II) complexes³⁶. For octahedral Ni(II) ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ transition occurs in the near IR region, which is seldom observed because it is in an inconvenient region³⁷. The Copper complexes (4-6) showed a d-d transition a t(410-650) nm for the ${}^2E_g \rightarrow {}^2T_{2g}$ transition of the d^9 configuration of Cu(II) in an octahedral environment. The Iron complexes (7-9) showed a d-d transtion at (390-600) nm for the ${}^2E_g \rightarrow {}^2T_{2g}$ for d^6 configuration of Fe(II) in an octahedral environment. On the basis of elemental analysis magnetic moment and conductance measurements, UV Visible spectra, infrared spectra and other physical properties the suggested structure of the complexes are octahedral in nature as in Fig.4.1.

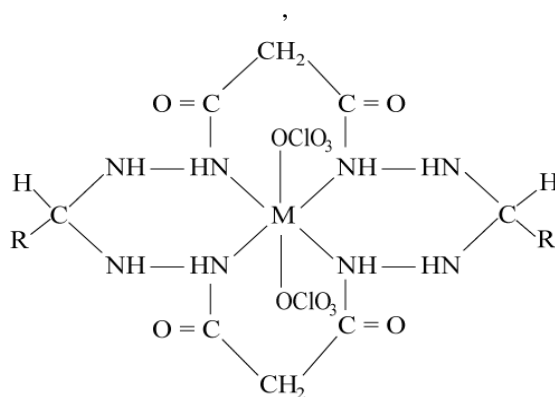


Fig. 4.1

M = Ni(II), where R=H(1), CH₃(2), C₆H₅CH=CH₂(3)

M = Cu(II), where R=H(4), CH₃(5), C₆H₅CH=CH₂(6)

M = Fe(II), where R=H(7), CH₃(8), C₆H₅CH=CH₂(9)

Table-4.1: Elemental Analytical data of compounds (1-9)

No	Compounds	% C		%H		%N	
		Calculated	Found	Calculated	Found	Calculated	Found
1	[Ni(C ₈ H ₁₆ N ₈ O ₄)(ClO ₄) ₂]	17.55	17.14	2.92	2.15	20.52	20.15
2	[Ni(C ₁₀ H ₂₀ N ₈ O ₄)(ClO ₄) ₂]	20.93	20.83	3.48	3.40	19.53	19.50
3	[Ni(C ₂₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	38.42	38.62	3.73	3.53	14.94	14.89
4	[Cu(C ₈ H ₈ N ₄ O ₁₆)(ClO ₄) ₂]	17.45	17.55	2.90	2.81	20.36	20.45
5	[Cu(C ₁₀ H ₂₀ N ₈ O ₄)(ClO ₄) ₂]	20.74	20.63	3.45	3.54	19.36	19.39
6	[Cu(C ₂₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	38.17	38.26	3.71	3.61	14.84	14.79
7	[Fe(C ₈ H ₁₆ N ₈ O ₄)(ClO ₄) ₂]	17.68	17.57	2.94	2.87	20.63	20.51
8	[Fe(C ₁₀ H ₂₀ N ₈ O ₄)(ClO ₄) ₂]	16.83	16.79	2.80	2.91	19.63	19.71
9	[Fe(C ₂₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	38.56	38.68	3.74	3.85	14.99	15.09

Table-4.2: Magnetic moment data of compounds (1-9). T = 301^ok

No	Compounds	Sample length, <i>l</i> In cm	Weight Of the Sample, <i>m</i> in gm	Susceptibility Of the empty tube, <i>R</i> ₀	Susceptibility of the sample with test tube, <i>R</i>	Mass susceptibility, $\chi_g \times 10^{-6}$ C.G.S. unit	Molecular weight, <i>M</i>	Molar susceptibility $\chi_g \times 10^{-6}$ C.G.S. unit	μ_{eff} B.M.
1	[Ni(C ₈ H ₁₆ N ₈ O ₄)(ClO ₄) ₂]	3.4	0.0594	-65	-15	5.97	545.69	3.258	2.89
2	[Ni(C ₁₀ H ₂₀ N ₈ O ₄)(ClO ₄) ₂]	2.9	0.0466	-60	-8	6.750	573.2	3.874	3.14
3	[Ni(C ₂₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	3.8	0.0346	-42	-20	5.04	749.6	3.77	2.82
4	[Cu(C ₈ H ₁₆ N ₄ O ₁₆)(ClO ₄) ₂]	2.9	0.0794	-59	-32	2.057	546.806	1.124	1.77
5	[Cu(C ₁₀ H ₂₀ N ₈ O ₄)(ClO ₄) ₂]	3.3	0.0626	-38	-31	0.769	578.5	0.442	1.24
6	[Cu(C ₂₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	2.6	0.093	-67	-66	0.583	754.5	0.439	1.33
7	[Fe(C ₈ H ₁₆ N ₈ O ₄)(ClO ₄) ₂]	4.5	0.0493	-79	-9	13.32	542.85	7.27	4.17
8	[Fe(C ₁₀ H ₂₀ N ₈ O ₄)(ClO ₄) ₂]	4.2	0.0405	-75	-8	14.49	570.36	8.27	4.46
9	[Fe(C ₂₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	4.3	0.0456	-77	-12	12.79	746.76	9.55	4.80

Table-4.3: Analytical data and other physical properties of compounds (1-9)

No	Compounds	% yield	Colour	Melting Point ^o C	M%		Molar Conductance ohm ⁻¹ cm ² mol ⁻¹
					Calculated	Found	
1	[Ni(C ₈ H ₁₆ N ₈ O ₄)(ClO ₄) ₂]	80	Light blue	270 (d)	10.73	10.83	3.23
2	[Ni(C ₁₀ H ₂₀ N ₈ O ₄)(ClO ₄) ₂]	60	Off white	255 (d)	10.22	9.99	1.63
3	[Ni(C ₂₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	75	Yellow	250 (d)	7.82	7.92	2.63
4	[Cu(C ₈ H ₈ N ₄ O ₁₆)(ClO ₄) ₂]	70	Olive green	200 (d)	11.53	11.98	1.99
5	[Cu(C ₁₀ H ₂₀ N ₈ O ₄)(ClO ₄) ₂]	65	Green	245 (d)	10.97	10.54	4.13
6	[Cu(C ₂₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	80	Yellow	230 (d)	8.15	8.01	2.63
7	[Fe(C ₈ H ₁₆ N ₈ O ₄)(ClO ₄) ₂]	80	White	210 (d)	10.28	10.02	1.29
8	[Fe(C ₁₀ H ₂₀ N ₈ O ₄)(ClO ₄) ₂]	70	Red	240 (d)	9.79	9.01	2.63
9	[Fe(C ₂₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	65	Prussian blue	235 (d)	7.47	6.93	3.14

Table-4.4: UV-visible absorption maxima of compounds (1-9)

No	Compounds	λ max (n.m)
1	[Ni(C ₈ H ₁₆ N ₈ O ₄)(ClO ₄) ₂]	450, 550
2	[Ni(C ₁₀ H ₂₀ N ₈ O ₄)(ClO ₄) ₂]	400, 600
3	[Ni(C ₂₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	410, 650
4	[Cu(C ₈ H ₈ N ₄ O ₁₆)(ClO ₄) ₂]	400, 570
5	[Cu(C ₁₀ H ₂₀ N ₈ O ₄)(ClO ₄) ₂]	400, 550
6	[Cu(C ₂₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	410, 650
7	[Fe(C ₈ H ₁₆ N ₈ O ₄)(ClO ₄) ₂]	410, 575
8	[Fe(C ₁₀ H ₂₀ N ₈ O ₄)(ClO ₄) ₂]	400, 550
9	[Fe(C ₂₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	390, 600

Table-4.5: Important infrared spectral bands of compounds (1-9)

No	Compounds	V(C-H)cm ⁻¹	V(C=O)cm ⁻¹	V(NH)cm ⁻¹	V(M-N)cm ⁻¹	V(ClO ₄)cm ⁻¹
1	[Ni(C ₈ H ₁₆ N ₈ O ₄)(ClO ₄) ₂]	2920	1674	3230	408	1145 1089 626
2	[Ni(C ₁₀ H ₂₀ N ₈ O ₄)(ClO ₄) ₂]	2972	1662	3265	412	1120 1087 626
3	[Ni(C ₂₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	2930	1649	3207	404	1130 1080 688
4	[Cu(C ₈ H ₈ N ₄ O ₁₆)(ClO ₄) ₂]	2930	1654	3219	412	1143 1087 626
5	[Cu(C ₁₀ H ₂₀ N ₈ O ₄)(ClO ₄) ₂]	2925	1654	3219	407	1143 1089 628
6	[Cu(C ₂₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	2950	1651	3207	404	1120 1082 617
7	[Fe(C ₈ H ₁₆ N ₈ O ₄)(ClO ₄) ₂]	2965	1678	3207	407	1109 1089 626
8	[Fe(C ₁₀ H ₂₀ N ₈ O ₄)(ClO ₄) ₂]	2916	1678	3265	405	1120 979 613
9	[Fe(C ₂₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	2918	1678	3207	412	1149 1082 688

Antibacterial Activity Testing

It has been observed that some drug increases the activity when administered as metal complexes or their metal chalets. The antibacterial activity of the metal complexes **1**, **3**, **5** and other complexes are recorded against fourteen pathogenic bacteria viz. *Salmonella-17*, *Klebsilla*, *Shigella dysenteriae*, *Shigella shiga*, *Shigella boydii*, *Shigella sonnei*, *Shigella flexneri*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella*, *Bacillus megaterium*, *Sarcina lutea*, *Staphylococcus aureus*, *Bacillus cereus* And the result is given in (Table 5.1-5.2) the complex **5** showed the most activities above fourteen pathogenic bacteria as shown (Fig 5.0). It is evident from all the tables that the under investigation showed the most activity compared to the complex **1**, **3**, **5**. The malanodihydrized complexes **1**, **3** and **5** have shown good activity against the above fourteen pathogenic bacteria as seen in (Table 5.1-5.2). The complex **1** showed the best activity against *E. Coli*, *Sarcina lutea* and less activity against *Klebsilla*. The complex **3** showed the best activity *Salmonella-17*, *Shigella shiga*, *Shigella sonnei* and less activity against *Pseudomonas aeruginosa*. The complex **5** showed the best activity against *Bacillus cereus* and less activity against *Shigella boydii* the complex **2**, **4**, **6**, are not showed good activities against the above fourteen pathogenic bacteria. The good activity against *Bacillus cereus* and less activity *Shigella dysenteriae* and other bacteria was not seen. Similarly the complex **2** showed good activities *Shigella shiga* and less activity against *Bacillus megatrium* and other bacteria was not seen activities. The complex **4** showed good activities *Salmonella* and less activities against *shigella sonnei* and other bacteria was not seen activities. The complex **6** showed good activities *Shigella dysenteriae* and less activities against *Shigella boydii*, *Bacillus megaterium* and other bacteria was not seen activities. All the result are compared with the standard compound, kanamycin as seen in the Table (5.1-5.2) the ligand malanodihyrazide (C₃H₈N₄O₂) did not show any activities against the above fourteen pathogenic bacteria. From here it is concluded that the complex **1**, **3** and **5** showed good activities against the fourteen pathogenic bacteria as compared to the standard compound, kanamycin. It is evident that the ligand malanodihyrazide did not show any activity.

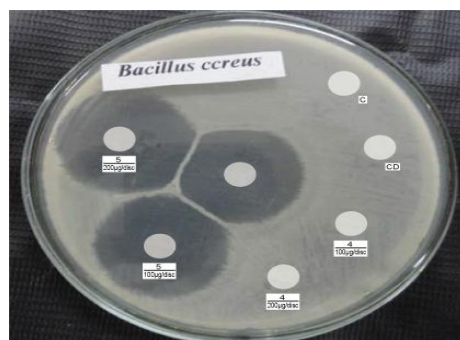


Fig: 5.0: Photographic representation of zone of inhibition of The complexes 4, 5 the standard compound kanamycin against *Bacillus cereus*.

Table-5.1

Name of microorganisms	Diameter of zone of inhibition (mm)									
	Complex 01			Complex 02			Complex 03			Stand 30
Done	200µg /disc	100µg /disc	50µg /disc	200µg /disc	100µg /disc	50µg /disc	200µg /disc	100µg /disc	50µg /disc	KAN
Gram negative bacteria										
1. <i>Salmonella-17</i>	-	-	-	4	2	-	17	8	-	20
2. <i>Klebsilla</i>	18	8	-	-	-	-	-	-	-	20
3. <i>Shigella dysenteriae</i>	8	-	-	8	6	-	-	-	-	20
4. <i>Shigella Shiga</i>	-	-	-	8	6	-	20	16	-	20
5. <i>Shigella boydii</i>	22	14	-	-	-	-	-	-	-	20
6. <i>Shigella sonnei</i>	-	-	-	-	-	-	18	14	-	20
7. <i>Shigella flexneri</i>	-	-	-	-	-	-	-	-	-	20
8. <i>Escherichia coli</i>	18	15	-	6	2	-	-	-	-	20
9. <i>Pseudomonas aeruginosa</i>	-	-	-	20	6	-	20	6	-	20
10. <i>Salmonella</i>	-	-	-	-	-	-	-	-	-	20
Gram negative bacteria										
11. <i>Bacillus megaterium</i>	-	-	-	-	-	-	-	-	-	20
12. <i>Sarcina lutea</i>	26	22	-	-	-	-	10	6	-	20
13. <i>Staphylococcus aureus</i>	-	-	-	-	-	-	19	16	-	20
14. <i>Bacillus cereus</i>	-	-	-	-	-	-	-	-	-	20

Table-5.2

Name of microorganisms	Diameter of zone of inhibition (mm)									
	Complex 04			Complex 05			Complex 06			Stand 30
Done	200µg /disc	100µg /disc	50µg /disc	200µg /disc	100µg /disc	50µg /disc	200µg /disc	100µg /disc	50µg /disc	KAN
Gram negative bacteria										
1. <i>Salmonella-17</i>	8	4	-	-	-	-	-	-	-	20
2. <i>Klebsilla</i>	-	-	-	-	-	-	-	-	-	20
3. <i>Shigella dysenteriae</i>	-	-	-	-	-	-	-	-	-	20
4. <i>Shigella Shiga</i>	-	-	-	-	-	-	-	-	-	20
5. <i>Shigella boydii</i>	-	-	-	18	16	-	8	6	-	20
6. <i>Shigella sonnei</i>	-	-	-	-	-	-	-	-	-	20
7. <i>Shigella flexneri</i>	-	-	-	-	-	-	8	6	-	20
8. <i>Escherichia coli</i>	-	-	-	-	-	-	-	-	-	20
9. <i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-	-	20
10. <i>Salmonella</i>	10	8	-	18	14	-	-	-	-	20
Gram negative bacteria										
11. <i>Bacillus megaterium</i>	-	-	-	-	-	-	8	-	-	20
12. <i>Sarcina lutea</i>	-	-	-	-	-	-	-	-	-	20
13. <i>Staphylococcus aureus</i>	8	2	-	-	-	-	-	-	-	20
14. <i>Bacillus cereus</i>	10	-	-	24	20	-	-	-	-	20

The present work also determined the minimum inhibitory concentration of the more active complexes 1, 3, 5 by a serial dilution method. The tube of broth medium (1mL) containing graded doses of sample were incubated with the test organisms. After suitable incubation growth occurred in these inhibitory tubes, where the concentration of the sample was below the inhibitory level, the culture became turbid (cloudy). The growth of the microorganisms was not observed above the inhibitory level and the growth of the microorganisms was not observed above the inhibitory level and the tubes remained clear. The minimum inhibitory results are furnished in Table-5.3.

Test organism	Complex 1	Complex 3	Complex 5
	MIC(µg/ml)		
<i>Salmonella-17</i>	64	128	64
<i>Klebsilla</i>	64	128	64
<i>Shigella dysenteriae</i>	32	128	64
<i>Shigella Shiga</i>	64	64	64
<i>Shigella boydii</i>	64	128	64
<i>Shigella sonnei</i>	128	128	64
<i>Shigella flexneri</i>	64	128	128
<i>Escherichia coli</i>	64	128	64
<i>Pseudomonas aeruginosa</i>	64	128	64
<i>Salmonella</i>	64	128	64
<i>Bacillus megaterium</i>	64	128	64
<i>Sarcina lutea</i>	64	64	64
<i>Staphylococcus aureus</i>	64	128	32
<i>Bacillus cereus</i>	64	128	64

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