

The Synthesized of Macro Cyclic Complex Compounds of Cd (Ii) Containing A Ligand Having Tetraoxo Tetrahydrazin Moity On Some Pathogenic Bacteria

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ABSTRACT : *The macrocyclic complex compounds of Cd(II) containing a ligand having tetraoxotetrahydrazin moiety are synthesized by template condensation of malonodihydrazide (C₃H₈N₄O₂) with different aldehydes. The complexes are characterized on the basis of elemental analysis, UV-visible & IR spectroscopy, GA, DTA, 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 moiety. 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

The chemistry of synthetic macrocyclic ligands can be divided into two broad divisions. Firstly there are the cyclic polyether's 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 cyclam 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. The recent article describing the use of [Cu-perchlorate contain complex] as a color indicator for solvent parameters¹⁰ 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"¹¹ 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 successful¹². Such compounds must be handled with great care¹³, if at all. The complex [Ag(INHSAL)₂(ClO₄)] and [Ag(INHHAP)₂ClO₄] have been synthesis¹⁴ 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.¹⁵

II. BIOLOGICAL ACTIVITY OF SOME IMPORTANT COMPOUNDS

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 system¹⁶. 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 outline of the chemistry and biological effects of some of the essential and polluting elements is given below.

A number of metal complexes and ligands have been shown to be chemically useful in a 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. Haemocyanin is found only in molluscs and arthropods. Inorganic chemistry has been interested in developing suitable copper complexes which would mimic some of the properties of haemocyanin.¹⁷

Aim of the Present Work : In the recent years considerable attention has been given to the synthesis of macrocyclic complexes¹⁸. These complex compounds have been used as a model system of biologically important materials, such as porphyrin and corins. Some of the macrocyclic ligands cannot be easily prepared from the reactants¹⁹. In that case the complex compounds could be synthesised by template method. The desired macrocyclic ligand can be isolated by stripping the complex compounds²⁰. Macrocyclic tetraaza complex of Ni²⁺ act as catalyst to reduce CO₂ to CO and Fe²⁺, Mn³⁺ porphyrins have been most commonly studied catalyst.²¹

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 rapidly increasing importance of macrocyclic ligands 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 Cd(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, Klebsiella, 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.

III. EXPERIMENTAL

The ligand precursor, malonodihydrazide was prepared by the literature procedure²². 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, TGA, DTA. 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 complexometrically using EDTA and DMG after fuming the complexes with sulfuric acid²². Melting points were determined on an electro thermal melting point apparatus (model no. AZ 6512).

IV. PREPARATION OF MACROCYCLIC COMPLEXES.

PREPARATION OF [Cd(C₈H₁₆N₈O₄)(ClO₄)₂] COMPLEX 1. : To the aqueous malonodihydrazide, C₃H₈N₄O₂ (0.792 g, 6 mmol in 10 mL water) formaldehyde solution (0.48 g, 6 mmol 37%) was added. To the above solution Cadmium(II) perchlorate hexahydrate (2.0829 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 CaCl₂. The melting point of the compound was 225°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, 3, 4, 5 and 6 using acetaldehyde, crotonaldehyde, cinnamaldehyde, benzaldehyde and butanaldehyde were the reaction mixture was refluxed for 4, 5, 3, 7 and 10 hours respectively.,

V. RESULT AND DISCUSSION

MACROCYCLIC COMPLEXES OF Cd (II) : Reactions of malonodihydrazide with Cd(II) perchlorate hexahydrate in presence of formaldehyde, acetaldehyde, crotonaldehyde, cinnamaldehyde, benzaldehyde and butanaldehyde give some 16 member macrocyclic complex as described above. Complexes (1-6) 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 (1-6) are shown in Table 4.1.

The conductance values of the complexes suggested that they are non-electrolytic in nature²⁴. The infrared spectra of the complexes (1-6) are shown as spectral data (Table 4.4) of the complexes showed a strong and broad band at (3250-3270) cm^{-1} which is assigned for the $\nu(\text{NH})$ stretching²⁵. Due to coordination the $\nu(\text{N-H})$ stretching of the amide group goes to the higher field at (3250, 3270) cm^{-1} region as compared to the starting material malonodihydrazide²⁶. 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 (3250, 3213, 3270) cm^{-1} . The bands at (3250, 3270) cm^{-1} for the asymmetric and symmetric $\nu(\text{N-H})$ stretching of the terminal- NH_2 moiety and 3261 cm^{-1} for amidic (N-H) group]. The complexes showed a broad band at (2920-3070) cm^{-1} is suggested for the $\nu(\text{C-H})$ stretching of aliphatic moiety³². The complexes showed a strong band at (1640-1680) cm^{-1} which represent the $\nu(\text{C=O})$ of NH-NH-CO- CH_2 moiety²⁷. Three or four band at (621-1180) 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 atom²⁸. A medium band at (406-430) cm^{-1} region is tentatively attributed to the $\nu(\text{M-N})$ mode^{29,30}. indicating the coordination of the ligand to the metal through the nitrogen atom. The magnetic moment measurement data (Table 4.3) of the Cd(II) complexes (1-6) showed (1.59-1.78) B.M. These values correspond to no unpaired electrons of Cd(II) d^{10} system suggest the octahedral environment of the complexes which are consistent with the literature value¹. The elemental analyses (C, H and N) (Table 4.2) and metal estimation data (Table 4.3) of the complexes are consistent with the proposed formula. The UV-visible spectra of the complexes (1-6) are shown (Table 4.5) band at 320,480 nm, (1-6) at represent the d-d transition of ${}^4A_{2g}(\text{F}) \rightarrow {}^4T_{1g}(\text{F})$, ${}^4A_{2g}(\text{F}) \rightarrow {}^4T_{1g}(\text{P})$, which suggested the octahedral geometry of the Cd(II) complexes^{31,32}.

Thermal studies: The thermal properties of metal(II) complexes were investigated by thermo grams TGA,DTA) and are shown in (Fig..) and the corresponding thermal analysis is presented in (Tabl.4.6). In the case of complex (I) (F.g 01) the decomposition occurs in the (230-325)⁰C range. There is no mass loss up to 230⁰C. The first stage of decomposition starts at 230⁰C and end at 230⁰C with a corresponding weight loss 25%. Which is accompanied by endothermic effect in the DTA curve in the range 225⁰C which is accompanied by weight loss confirming the second stage of decomposition is observed at 225-350⁰C (60% wt. loss). meanwhile the DTA curve exhibits endothermic effect in the range 325⁰C which is accompanied by weight loss confirming. On the basis of elemental analysis magnetic moment and conductance measurements, thermal studies UV Visible spectra, infrared spectra and other physical properties the suggested structure of the complexes are octahedral in nature as in Fig.4.1.

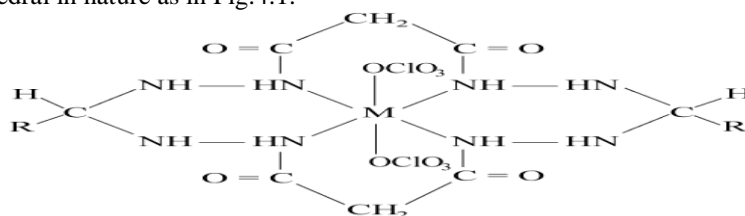


Fig. 4.1

M =Cd (II), where R=H(1), CH_3 (2), $\text{CH}_3\text{CH}=\text{CH}$ -(3) $\text{C}_6\text{H}_5\text{CH}=\text{CH}_2$ -(4), $\text{CH}_3-\text{CH}_2-\text{CH}_2$ -(5), $\text{C}_6\text{H}_5\text{CH}$ -(6)

TABLE- 4.1: Analytical Data and Other Physical Properties of Compounds (1-6)

No.	compounds	%Yield	Colour	Milting point ⁰ C	% M		Molar conductance $\text{ohm}^{-1}\text{cm}^2\text{mol}^{-1}$
					Calculated	Found	
1	$[\text{Cd}(\text{C}_8\text{H}_{16}\text{N}_8\text{O}_4)(\text{ClO}_4)_2]$	60	White	217	18.69	18.60	10.48
2	$[\text{Cd}(\text{C}_{10}\text{H}_{20}\text{N}_8\text{O}_4)(\text{ClO}_4)_2]$	70	Yellow	210	17.86	17.80	15.10
3	$[\text{Cd}(\text{C}_{14}\text{H}_{28}\text{N}_8\text{O}_4)(\text{ClO}_4)_2]$	70	Red	215	16.39	16.36	13.50
4	$[\text{Cd}(\text{C}_{14}\text{H}_{24}\text{N}_8\text{O}_4)(\text{ClO}_4)_2]$	60	Red	220	16.49	16.40	16.08
5	$[\text{Cd}(\text{C}_{24}\text{H}_{28}\text{N}_8\text{O}_4)(\text{ClO}_4)_2]$	70	Ashe	215	13.94	13.90	14.00
6	$[\text{Cd}(\text{C}_{20}\text{H}_{24}\text{N}_8\text{O}_4)(\text{ClO}_4)_2]$	75	Black	220	14.91	14.90	12.49

Table- 4.3: Magnetic moment data of compounds (1-6)

No.	Compounds	Sample length, l in cm	Weight of the sample, m in gm	Susceptibility of the empty tube, R ₀	Susceptibility of the sample with tube ,R	Mass Susceptibility x _g ×10 ⁻⁶ C.G.S.unit	Molecular weight M	Molar Susceptibility x _g ×10 ⁻⁶ C.G.S.unit	μ _{eff} B.M
1	[Cd(C ₈ H ₁₆ N ₈ O ₄)(ClO ₄) ₂]	2.2	0.0695	-48	-22	1.71	599	0.712	1.30
2	[Cd(C ₁₀ H ₂₀ N ₈ O ₄)(ClO ₄) ₂]	2.1	0.0692	-47	-23	1.51	627	0.712	1.30
3	[Cd(C ₁₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	2.2	0.0596	-40	-21	1.46	683	0.771	1.36
4	[Cd(C ₁₄ H ₂₄ N ₈ O ₄)(ClO ₄) ₂]	1.8	0.0559	-46	-24	1.47	679	0.896	1.46
5	[Cd(C ₂₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	1.8	0.0630	-42	-20	1.31	803	0.883	1.45
6	[Cd(C ₂₀ H ₂₄ N ₈ O ₄)(ClO ₄) ₂]	1.7	0.0589	-46	-23	1.38	751	1.036	1.57

Table- 4.4: important infrared spectral bands of compounds (1-6)

No.	Compounds	ν (C-H) cm ⁻¹	ν (C=O) cm ⁻¹	ν (N-H) cm ⁻¹	ν (M-N) cm ⁻¹	ν (ClO ₄) cm ⁻¹
1	[Cd(C ₈ H ₁₆ N ₈ O ₄)(ClO ₄) ₂]	3047	1664	3251	420	1180,1089,623
2	[Cd(C ₁₀ H ₂₀ N ₈ O ₄)(ClO ₄) ₂]	2920	1649	3282	430	1105,979,623
3	[Cd(C ₁₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	2960	1640	3250	416	1150,1060,620
4	[Cd(C ₁₄ H ₂₄ N ₈ O ₄)(ClO ₄) ₂]	2967	1650	3261	408	1160,1040,623
5	[Cd(C ₂₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	3040	1660	3254	409	1140,1080,621
6	[Cd(C ₂₀ H ₂₄ N ₈ O ₄)(ClO ₄) ₂]	3070	1680	3270	406	1170,1090,625

TABLE- 4.5: U.V- VISIBLE ADSORPTION MAXIMA OF COMPOUNDS (1-6)

No.	Compounds	λ max (n,m)
1	[Cd(C ₈ H ₁₆ N ₈ O ₄)(ClO ₄) ₂]	330
2	[Cd(C ₁₀ H ₂₀ N ₈ O ₄)(ClO ₄) ₂]	350
3	[Cd(C ₁₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	330
4	[Cd(C ₁₄ H ₂₄ N ₈ O ₄)(ClO ₄) ₂]	350
5	[Cd(C ₂₄ H ₂₈ N ₈ O ₄)(ClO ₄) ₂]	350
6	[Cd(C ₂₀ H ₂₄ N ₈ O ₄)(ClO ₄) ₂]	350

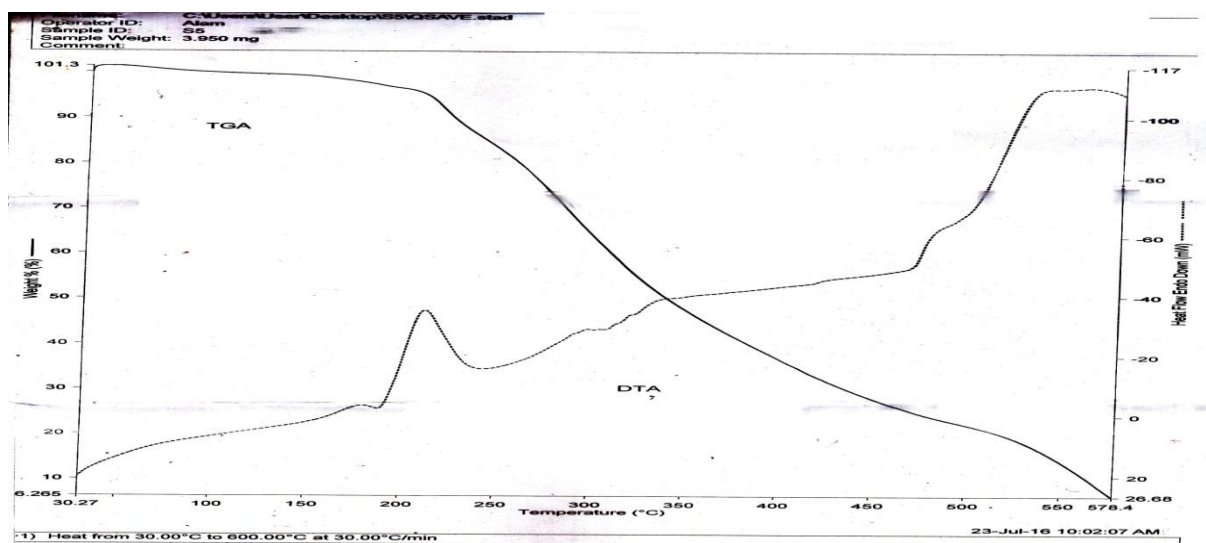


Fig.2:TGA,DTA

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 **1,2, 3, 4,5** 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 **1, 4** 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, 2, 3, 4,5** The malanodihydrized complexes **1, 3, 4** 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, *Shigella boydii, Escherichia, Sarcina luteacoli* and less activity against *Shigella dysenteriae*. The complex **2** showed the best activity, *Pseudomonas aeruginosa* and less activity against *Salmonella-17*. The complex **3** showed the best activity, *shigella sonnei, Shigella shiga* and less activity against *Shigella dysenteriae, Escherichia coli*. The complex **4** showed the best activity against *Shigella boydii* and less activity against *Shigella flexneri*. The complex **5** showed the best activity against *Bacillus megaterium* and less activity against *Sarcinalutea*. The complex **6** are not showed good activities against the above fourteen pathogenic bacteria. The complex **1** showed good activities *Shigella boydii* and less activity against, *Shigella dysenteriae*. The complex **2** showed good activities *Pseudomonas aeruginosa* and less activities against *Salmonella-17*. The complex **3** showed good activitie *Shigella shiga* and less activities against *Klebsilla*. All the result are compared with the standard compound, kanamycin as seen in the Table (5.1-5.2) the ligand malanodihyrazide ($C_3H_8N_4O_2$) did not show any activities against the above fourteen pathogenic bacteria. From here it is concluded that the complex **1, 3** and **4** 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 3 the standard compound kanamycin against Shigallsonnei

Table-5.1

Name of microorganisms	Diameter of zone of inhibition (mm)									
	Complex01			Complex02			Complex03			Stand 30
Name of test sample	200µg /disc	100µg /disc	50 µg /disc	200µg /disc	100µg /disc	50 µg /disc	200µg /disc	100µg /disc	50 µg /disc	KAN
Gram negative bacteria										
1. <i>Salmonella-17</i>	15	9	3	12	6	2	17	8	4	20
2. <i>Klebsilla</i>	16	7	3	10	5	2	8	2	-	20
3. <i>Shigella dysenteriae</i>	12	5	2	14	6	3	8	5	2	20
4. <i>Shigella shiga</i>	16	7	4	13	6	3	18	12	4	20
5. <i>Shigella boydii</i>	18	10	4	10	5	2	10	4	2	20
6. <i>Shigella sonnei</i>	16	7	3	15	7	3	18	14	4	20
7. <i>Shigella flexneri</i>	15	6	2	14	7	2	7	3	2	
8. <i>Escherichia coli</i>	18	10	5	16	7	3	8	4	2	20
9. <i>Pseudomonas aeruginosa</i>	16	8	4	18	8	3	16	8	4	20
10. <i>Salmonella</i>	15	7	3	12	6	3	10	5	2	20
Gram positive bacteria										

11. <i>Bacillus megaterium</i>	16	10	4	15	7	3	15	6	3	20
12. <i>Sarcina lutea</i>	18	12	5	13	6	2	10	6	2	20
13. <i>Staphylococcus aureus</i>	15	6	3	14	6	3	17	9	6	20
14. <i>Bacillus cereus</i>	16	8	4	12	6	2	10	5	2	20

Table-5.2

Name of microorganisms	Diameter of zone of inhibition (mm)									
Name of test sample	Complex04			Complex05			Complex06			Stand 30
Done	200µg /disc	100µg /disc	50 µg /disc	200µg /disc	100µg /disc	50 µg /disc	200µg /disc	100µg /disc	50 µg /disc	KAN
Gram negative bacteria										
1. <i>Salmonella-17</i>	16	8	3	15	6	2	-	-	-	20
2. <i>Klebsilla</i>	10	4	2	16	8	3	-	-	-	20
3. <i>Shigella dysenteriae</i>	15	7	2	14	7	2	-	-	-	20
4. <i>Shigella shiga</i>	16	7	3	14	7	2	-	-	-	20
5. <i>Shigella boydii</i>	18	9	4	16	8	3	8	3	-	20
6. <i>Shigella sonnei</i>	12	5	2	15	7	3	-	-	-	20
7. <i>Shigella flexneri</i>	10	4	2	12	6	2	8	3		20
8. <i>Escherichia coli</i>	15	4	2	14	6	3	-	-	-	20
9. <i>Pseudomonas aeruginosa</i>	15	9	3	15	7	3	-	-	-	20
10. <i>Salmonella</i>	12	8	4	18	8	3	-	-	-	20
Gram positive bacteria										
11. <i>Bacillus megaterium</i>	12	6	3	16	7	3	8	-		20
12. <i>Sarcina lutea</i>	14	7	2	12	5	2	-	-	-	20
13. <i>Staphylococcus aureus</i>	13	6	2	14	7	3	-	-	-	20
14. <i>Bacillus cereus</i>	10	4	2	16	8	3	-	-	-	20

The present work also determined the minimum inhibitory concentration of the more active complexes 1, 3, 4 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.

Table-5.3.

Test organism	Complex 1	Complex 2	Complex 3	Complex 4	Complex 5
	MIC(µg/mL)				
<i>Salmonella-17</i>	32	32	64	32	32
<i>Klebsilla</i>	32	32	64	32	32
<i>Shigella dysenteriae</i>	16	32	64	32	32
<i>Shigella shiga</i>	32	32	64	32	32
<i>Shigella boydii</i>	32	32	64	32	32
<i>Shigella sonnei</i>	32	16	64	16	32
<i>Sigella flexneri</i>	32	32	64	32	32
<i>Escherichia coli</i>	32	32	64	32	16
<i>Pseudomonas aeruginosa</i>	32	32	64	32	32

<i>Salmonella</i>	32	32	64	32	32
<i>Bacillus megaterium</i>	64	32	64	32	32
<i>Sarcina lutea</i>	32	64	64	64	64
<i>Staphylococcus aureus</i>	32	32	32	32	32
<i>Bacillus cereus</i>	32	32	64	32	32

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