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# The Influence of a New-Synthesized Complex Compounds of Co(II), Zn(II) and Cd(II) Containing a Ligand having Tetraoxotetrahydrazin Moiety on some Pathogenic Bacteria.

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Some macrocyclic complex compounds of Co(II), Zn(II) and Cd(II) containing a ligand having tetraoxotetrahydrazin moiety are synthesized by template condensation of malonodihydrazide ( $C_3H_8N_4O_2$ ) with different 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 five 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.

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*Keyword:* Ligand, Tetraoxotetrahydrazin, Malonodihydrazide ( $C_3H_8N_4O_2$ ), kanamycin, pathogenic, porphyrin, corrin, Haemocynin.

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### 1. 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 (Fig-1) is a typical example<sup>[1]</sup>. Ligands of this general category have received much recent attention because of their unusual behavior towards a range of non-transition metal ions<sup>[2]</sup>. Few studies involving transition metal ions have

been reported<sup>[3]</sup> and it is evident that the majority of such polyether ligands show a limited tendency to form stable complexes with these ions<sup>[4]</sup>.

The coordination chemistry of hydrazones is an intensive area of study and numerous transition metal complexes of these ligands have been investigated<sup>[5]</sup>. 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 species<sup>[6]</sup>. Coordination compounds derived from arylhydrazones have been reported because of their anti-tuberculosis, antimicrobial and corrosion inhibitors<sup>[7]</sup>. The chemistry and complexation properties of macrocyclic dioxotetraamines were investigated<sup>[8]</sup>. 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 deprotonated<sup>191</sup>.

### 1.1 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 books<sup>1101</sup>. 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"<sup>1111</sup>, 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 B<sub>12</sub> coenzyme<sup>1121</sup>. 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 recently<sup>1131</sup>.

### 1.2 Coordination Complex of Perchlorate Ion

The recent article describing the use of [Cu(tmen)(arac)]ClO<sub>4</sub> as a color indicator for solvent parameters<sup>1141</sup> 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"<sup>1151</sup> 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(H<sub>2</sub>O)<sub>3</sub>(ClO<sub>4</sub>)<sub>2</sub> detonated under a slight impact while attempts to repeat the detonation with other samples were not successful<sup>1161</sup>. Such compounds must be handled with great care<sup>1171</sup>, if at all.

The complex [Ag(1NHSAL)<sub>2</sub>(ClO<sub>4</sub>)] and [Ag(INHHAP)<sub>2</sub>ClO<sub>4</sub>] have been synthesized<sup>1181</sup> by adding ligand dropwise and mixed with metal perchlorate solution in the 2:1 molar ratio, (where ligand 1NHSAL = 2-hydroxybenzaldehyde) 1 NHHAP=2hydroxy acetophenone. On the basis of IR spectra showed a band at (1090-620) cm<sup>-1</sup> regions these are assignable that perchlorate coordinate to the metal<sup>1191</sup>.

### 1.3 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 system<sup>1201</sup>. 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 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 O<sub>2</sub> 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<sup>1211</sup>.

### 1.4 Aim of The Present Work

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

In nature a lot of macrocyclic compounds are known (e.g. haemoglobin, myoglobin, vitamin B<sub>12</sub> coenzymes where the metal atoms are Fe, Co and the basic macrocyclic unit is porphyrin but have different functional groups to the macrocycles). The chemical properties of some macrocyclic compounds resemble those of antibiotics<sup>[22]</sup>. For instance; macrolactones resemble the natural metabolites such as nonactin and monactin.

In the recent years considerable attention has been given to the synthesis of macrocyclic complexes<sup>[23]</sup>. 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 reactants<sup>[24]</sup>. In that case the complex compounds could be synthesised by the template method. The desired macrocyclic ligand can be isolated by stripping the complex compounds<sup>[25]</sup>. Macrocyclic tetraaza complexes of Ni<sup>2+</sup> act as catalysts to reduce CO<sub>2</sub> to CO and Fe<sup>2+</sup>, Mn<sup>3+</sup> porphyrins have been most commonly studied catalysts<sup>[26]</sup>.

In view of the extensive use as drugs and significant pharmacological activities of macrocyclic complexes and their derivatives, it is desired to synthesise macrocyclic complexes of Co (II), Zn (II) and Cd (II). The synthesised 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:

a. Firstly, synthesis of some new macrocyclic complexes by the reactions of

malonodihydrazide with Co(II), Zn(II) and Cd(II) perchlorate in the presence of formaldehyde, acetaldehyde, cinnamaldehyde and crotonaldehyde will be characterised by elemental analysis, UV visible and IR spectral analysis, magnetic moment and conductance measurements and some other physical properties.

b. Secondly, study of antibacterial activity of the synthesised complexes (some test organisms such as, *Klebsilla*, *Shigella flexneri*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus cereus*) including the investigation of minimum inhibitory concentration of the complexes.

### 2. Experimental

The ligand precursor, malonodihydrazide was prepared by the literature procedure<sup>[27]</sup>. 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<sup>-1</sup> 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 complexometrically using EDTA and DMG after fuming the complexes with sulfuric acid. Melting points were determined on an electro thermal melting point apparatus (model no. AZ 6512).

### 3. Preparation of Complexes (1-6)

To the aqueous malonodihydrazide (C<sub>3</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>) (0.528 g, 4 mmol in 10 mL water) solution, formaldehyde (0.320 g, 4 mmol 37%) solution was added. To the above mixture, cadmium (II) perchlorate hexahydrate (0.839 g, 2 mmol in 10 mL water) solution was added and the whole mixture was refluxed with constant stirring for two hours and cooled down. A white precipitate

was formed immediately. The precipitate was filtered off on a buckner funnel. Then the product was washed three times with ethanol and dried in a vacuum desiccator over anhydrous  $\text{CaCl}_2$ . The melting point of the compound was  $270^\circ\text{C}$  (decompose) and yield was 1.290 g (76%). 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 and 4 using acetaldehyde, cinnamaldehyde and crotonaldehyde were the reaction mixture was refluxed for 5, 8 and 12 hours respectively. Similarly the complexes (5-6) were Synthesized by the reaction of malonodihydrazide, Copper(II) Perchlorate and Corresponding aldehydes (viz., formal dehyde, cinnamaldehyde) respectively. The complexes (5-6) were prepared by refluxing for 4 and 7 hours respectively.

## 4. Result And Discussion

### 4.1 Macrocylic Complexes of M (II)

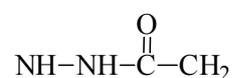
Reactions of malonodihydrazide with metal (II) perchlorate hexahydrate (where  $\text{M}=\text{Ni(II)}$ ,  $\text{Cu(II)}$ ,  $\text{Fe(II)}$ ) in presence of formaldehyde, acetaldehyde and cinnamaldehyde give some 16 member macrocylic complex as described above

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 3. The conductance values of the complexes suggested that they are non-electrolytic in nature. UV-visible absorption maxima of compounds (1-9) are shown in Table 4

The infrared spectra of the complexes (1-6) are shown as spectral data (Table 5) of the complexes showed a strong and broad band at  $(3246-3265)\text{ cm}^{-1}$  which is assigned for the  $\nu(\text{NH})$  stretching.

Due to coordination the  $\nu(\text{N-H})$  stretching of amide group goes to the higher frequency at  $(3222, 3261)\text{ cm}^{-1}$  region as compared to the starting material malonodihydrazide. In the

complexes the terminal- $\text{NH}_2$  group of malonodihydrazide condensed with aldehyde moiety form a new secondary -NH group which may 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- $\text{NH}_2$  moiety and  $3213\text{ cm}^{-1}$  for the  $\nu(\text{N-H})$  stretching of the amidic(N-H) group]. The complexes showed a broad band at  $(2912-3047)\text{ cm}^{-1}$  which may be assigned to the  $\nu(\text{C-H})$  stretching of aliphatic moiety. The complexes showed a strong band  $(1652-1685)\text{ cm}^{-1}$  which



represent the  $\nu(\text{C=O})$  of moiety. Three or four bands at  $(621-1143)\text{ cm}^{-1}$  region also indicated the  $\nu_1, \nu_2, \nu_3, \nu_4$  bands of  $(\text{ClO}_4^-)$  moiety. These stretching frequencies suggested the coordination of perchlorate to the metal through the O atom. A medium band at  $(416-443)\text{ cm}^{-1}$  region is tentatively attributed to the  $\nu(\text{M-N})$  mode indicating the coordination of the metal through the nitrogen atom.

The magnetic moment measurement data (Table 2) of the Cd(II) complexes (1-4) showed values of  $(0.02-0.03)$  B.M. These values correspond to no unpaired electron of Cd(II) system. The elemental analysis (C, H and N) (Table 1) and metal estimated data (Table 3) of the complexes are consistent with the proposed formula.

The UV-visible spectra of the complexes (1-4) are shown (Table 4). The complexes showed bands (Table 4.5) at 350, 400 nm, (1) at 350, 400 nm, (2) at 370, 550 nm, (3) and 350, 400 nm, (4) represent the d-d transition of  ${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{F})$ ,  ${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{P})$ , which suggested the octahedral geometry of the Cd(II) complexes.

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.1.

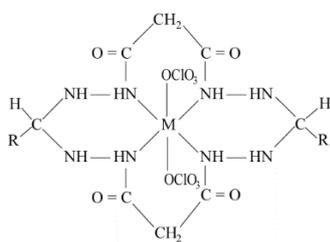


Fig. 1

Suggested structure of the Macrocyclic Complexes of M (II):

M = Cd(II), where R=H(1), CH<sub>3</sub>(2), C<sub>6</sub>H<sub>5</sub>CH=CH<sub>2</sub>(3), CH<sub>3</sub>CH=CH(4)

M = Co(II), where R=H(5), C<sub>6</sub>H<sub>5</sub>CH=CH<sub>2</sub>(6)

Table-1: Data for elemental analysis of compounds (1-6).

No.	Compounds	% C		% H		% N	
		Calculated	Found	Calculated	Found	Calculated	Found
1	[Cd(C <sub>8</sub> N <sub>8</sub> O <sub>4</sub> H <sub>16</sub> )(ClO <sub>4</sub> ) <sub>2</sub> ]	16.02	16.09	2.67	2.69	16.70	16.71
2	[Cd(C <sub>10</sub> N <sub>8</sub> O <sub>4</sub> H <sub>20</sub> )(ClO <sub>4</sub> ) <sub>2</sub> ]	19.12	19.14	3.19	3.20	17.86	17.79
3	[Cd(C <sub>24</sub> N <sub>8</sub> O <sub>4</sub> H <sub>28</sub> )(ClO <sub>4</sub> ) <sub>2</sub> ]	35.83	35.90	3.49	3.50	13.95	13.97
4	[Cd(C <sub>14</sub> N <sub>8</sub> O <sub>4</sub> H <sub>24</sub> )(ClO <sub>4</sub> ) <sub>2</sub> ]	24.74	24.76	3.53	3.44	16.49	16.30
5	[Co(C <sub>8</sub> N <sub>8</sub> O <sub>4</sub> H <sub>16</sub> )(ClO <sub>4</sub> ) <sub>2</sub> ]	17.58	17.67	2.93	2.94	20.51	20.50
6	[Co(C <sub>24</sub> N <sub>8</sub> O <sub>4</sub> H <sub>28</sub> )(ClO <sub>4</sub> ) <sub>2</sub> ]	38.40	38.50	3.73	3.75	14.93	14.97

Table-2: Magnetic moment data of compounds (1-6). T=301K

No	Compounds	Sample length, l in cm	Weight of the sample, m in gm	Susceptibility of the empty tube, R <sub>0</sub>	Susceptibility of the sample with test tube, R	Mass Susceptibility, χ <sub>g</sub> × 10 <sup>-6</sup> C.G.S. unit	Molecular weight, M	Molar Susceptibility, χ <sub>m</sub> × 10 <sup>-3</sup> C.G.S. unit	μ <sub>eff</sub> B.M.
1	[Cd(C <sub>8</sub> N <sub>8</sub> O <sub>4</sub> H <sub>16</sub> )(ClO <sub>4</sub> ) <sub>2</sub> ]	2.1	0.671	-42	-22	0.130	599.41	0.078	0.02
2	[Cd(C <sub>10</sub> N <sub>8</sub> O <sub>4</sub> H <sub>20</sub> )(ClO <sub>4</sub> ) <sub>2</sub> ]	3.1	0.851	-35	-20	0.114	627.41	0.071	0.02
3	[Cd(C <sub>24</sub> N <sub>8</sub> O <sub>4</sub> H <sub>28</sub> )(ClO <sub>4</sub> ) <sub>2</sub> ]	2.7	0.568	-40	-18	0.218	803.41	0.175	0.03
4	[Cd(C <sub>14</sub> N <sub>8</sub> O <sub>4</sub> H <sub>24</sub> )(ClO <sub>4</sub> ) <sub>2</sub> ]	2.4	0.634	-38	-25	0.103	679.41	0.697	0.02
5	[Co(C <sub>8</sub> N <sub>8</sub> O <sub>4</sub> H <sub>16</sub> )(ClO <sub>4</sub> ) <sub>2</sub> ]	3.8	0.2650	-52	-15	11.067	546	6.043	3.81
6	[Co(C <sub>24</sub> N <sub>8</sub> O <sub>4</sub> H <sub>28</sub> )(ClO <sub>4</sub> ) <sub>2</sub> ]	3.7	0.0187	-40	-20	8.255	750	6.191	3.86

**Table 3:** Analytical data and other physical properties of compounds (1-6).

No.	Compounds	% yield	Colour	Melting points °C	M%		Molar conductance $\text{Ohm}^{-1}\text{cm}^2\text{mol}^{-1}$
					Calculated	Found	
1	$[\text{Cd}(\text{C}_8\text{N}_8\text{O}_4\text{H}_{16})(\text{ClO}_4)_2]$	76	White	210(d)	17.941	18.760	2.0
2	$[\text{Cd}(\text{C}_{10}\text{N}_8\text{O}_4\text{H}_{20})(\text{ClO}_4)_2]$	58	Pale yellow	270(d)	17.064	17.928	3.18
3	$[\text{Cd}(\text{C}_{24}\text{N}_8\text{O}_4\text{H}_{28})(\text{ClO}_4)_2]$	51	Pale brown	200(d)	14.052	13.998	1.87
4	$[\text{Cd}(\text{C}_{14}\text{N}_8\text{O}_4\text{H}_{24})(\text{ClO}_4)_2]$	70	Offwhite	286(d)	16.021	16.555	1.33
5	$[\text{Co}(\text{C}_8\text{N}_8\text{O}_4\text{H}_{16})(\text{ClO}_4)_2]$	76	Light pink	290(d)	10.86	10.09	2.65
6	$[\text{Co}(\text{C}_{24}\text{N}_8\text{O}_4\text{H}_{28})(\text{ClO}_4)_2]$	58	Light brown	180(d)	7.86	6.95	1.24

d= The temperature at which the macrocyclic compound was decomposed.

**Table 4:** UV-visible absorption maxima of compounds (1-6).

No	compounds	$\lambda_{\text{max}}$ (nm)
1	$[\text{Cd}(\text{C}_8\text{N}_8\text{O}_4\text{H}_{16})(\text{ClO}_4)_2]$	350, 400
2	$[\text{Cd}(\text{C}_{10}\text{N}_8\text{O}_4\text{H}_{20})(\text{ClO}_4)_2]$	350, 400
3	$[\text{Cd}(\text{C}_{24}\text{N}_8\text{O}_4\text{H}_{28})(\text{ClO}_4)_2]$	370, 550
4	$[\text{Cd}(\text{C}_{14}\text{N}_8\text{O}_4\text{H}_{24})(\text{ClO}_4)_2]$	350, 400
5	$[\text{Co}(\text{C}_8\text{N}_8\text{O}_4\text{H}_{16})(\text{ClO}_4)_2]$	400, 500
6	$[\text{Co}(\text{C}_{24}\text{N}_8\text{O}_4\text{H}_{28})(\text{ClO}_4)_2]$	390, 550

**Table 5:** Important infrared spectral bands of compounds (1-6).

No	Compounds	$\nu(\text{C-H})\text{cm}^{-1}$	$\nu(\text{C=O})\text{cm}^{-1}$	$\nu(\text{NH})\text{cm}^{-1}$	$\nu(\text{M-N})\text{cm}^{-1}$	$\nu(\text{ClO}_4)\text{cm}^{-1}$
1	$[\text{Cd}(\text{C}_8\text{N}_8\text{O}_4\text{H}_{16})(\text{ClO}_4)_2]$	3261	3047	1685	1143,1087,626	443
2	$[\text{Cd}(\text{C}_{10}\text{N}_8\text{O}_4\text{H}_{20})(\text{ClO}_4)_2]$	3257	2979	1670	1120,1087,623	430
3	$[\text{Cd}(\text{C}_{24}\text{N}_8\text{O}_4\text{H}_{28})(\text{ClO}_4)_2]$	3222	3058	1652	1122,1074,621	416
4	$[\text{Cd}(\text{C}_{14}\text{N}_8\text{O}_4\text{H}_{24})(\text{ClO}_4)_2]$	3257	2974	1670	1122,979,623	430
5	$[\text{Co}(\text{C}_8\text{N}_8\text{O}_4\text{H}_{16})(\text{ClO}_4)_2]$	3257	3008	1676	1120,970,613	420
6	$[\text{Co}(\text{C}_{24}\text{N}_8\text{O}_4\text{H}_{28})(\text{ClO}_4)_2]$	3211	2902	1651	1153,1076,623	424

#### 4.2 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 2, 3, 6 and other complexes are recorded against five pathogenic bacteria viz.

*Klebsilla*, *Shigella flexneri*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus cereus*. And the results are given in (Table 6-7) the complex 7(3) showed the most activities above

five pathogenic bacteria as shown Table (Fig. 2). It is evident from all the tables that the under investigation showed the most activity compared to the complex 7(3).

The malanodihydazied complexe 7 shown good activity against the above five pathogenic bacteria as seen in (Table 6-7).

The good activity against *Bacillus ccreus* and less active *Klebsilla* and other bacteria was not seen. All the results are compared with the standard compound, Kanamycin as seen in the Table (5.1-5.6) the ligand malondihydrazide ( $C_3H_8N_4O_2$ ) did not show any activities against the above five pathogenic bacteria.

From here it is concluded that the complex 7(3) showed medium activities against one pathogenic bacteria (*Bacillus ccreus*) as compared to the standard compound, kanamycin. It is evident that the ligand malondihydrazide did not show any activity.

**Table-6:** The antibacterial activity of the metal complexes 2, 3 and 6 standard compound kanamycin(KAN) against five pathogenic bacteria :

Name of microorganisms	Diameter of zone of inhibition (mm)									
	[Cd(C10N8O4H20)(ClO4)2]			[Cd(C24N8O4H28)(ClO4)2]			[Co(C24N8O4H28)(ClO4)2]			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>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-	-	25
2. <i>Klebsilla</i>	-	-	-	-	-	-	-	-	-	20
3. <i>Shigella flexneri</i>	-	-	-	-	-	-	-	-	-	18
4. <i>Escherichia coli</i>	-	-	-	-	-	-	-	-	-	20
Gram positive bacteria										
5. <i>Bacillus cereus</i>	-	-	-	15	8	-	-	-	-	22

The present work also determined the minimum inhibitory concentration of the more active complex 3 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

The minimum inhibitory concentration for the complexes 7(3) was determined and shown in Table 8.



**Fig. 5.2:** Photographic representation of zone of inhabitation of the complex 7(3) and the standard compound kanamycin against *Bacillus ccreus*.

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-8.

**Table 8:** Minimum inhibitory concentration of  $[\text{Cd}(\text{C}_{24}\text{N}_8\text{O}_4\text{H}_{28})(\text{ClO}_4)_2]$  complex, **7(3)** against *Bacillus cereus*.

Test tube No	Nutrient broth medium added (mL)	Diluted solution of complex ( $\mu\text{g/mL}$ )	Inoculum added ( $\mu\text{L}$ )	Observation
1.	1	512	10	-ve
2.	1	256	10	-ve
3.	1	128	10	+ve
4.	1	64	10	+ve
5.	1	32	10	+ve
6.	1	16	10	+ve
7.	1	8	10	+ve
8.	1	4	10	+ve
9.	1	2	10	+ve
C <sub>s</sub>	1	512	0	-ve
C <sub>i</sub>	1	0	10	+ve
C <sub>M</sub>	1	0	0	-ve

\*The MIC of the complex **7** is 128  $\mu\text{g/mL}$

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