Synthesis, Characterization and Biological Activity Study of Some Macrocyclic Complexes of Co(II), Zn(II) and Cd(II)
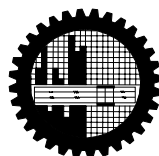
Submitted By

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CANDIDATE'S DECLARATION

It is hereby declared that this thesis or any part of it has not been submitted elsewhere for the award of any degree or diploma.

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Dedicated to

My

Family

Bangladesh University of Engineering and Technology, BUET, Dhaka.
Department of Chemistry

Certification of Thesis

A thesis on

SYNTHESIS, CHARECTERIZATION AND BIOLOGICAL ACTIVITY
STUDY OF SOME MACROCYCLIC COMPLEXES OF Co(II), Zn(II)
AND Cd(II)

SUBMITTED BY

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EXPERIMENTAL

LIST OF ABBREVIATION

1.	g	Gram
2.	cm	Centimeter
3.	cm^{-1}	Per Centimeter
4.	Fig	Figure
5.	i.e.	That is
6.	K	Degree Kelvin
7.	m.p.	Melting point
8.	mL	Milliliter
9.	mmol	Millimol
10.	No.	Number
11.	%	Percent
12.	Λ	Conductance
13.	χ_g	Mass susceptibility
14.	χ_m	Molar mass susceptibility
15.	λ_{max}	Absorption Maximum
16.	υ	Wavenumbers
17.	μg	Microgram
18.	IR	Infrared
19.	Equ	Equation
20.	B. M.	Bohr magnetron
21.	M	Moles/litre
22.	DMSO	Dimethylsulfoxide
23.	DMF	Dimethyl formamide

ABSTRACT

Macrocyclic chemistry is a growing area of research in inorganic and bioinorganic chemistry in view of its biological significance. The studies of macrocycles have undergone tremendous growth in recent years and their complexation chemistry with a wide variety of metal ions has been extensively studied. Macrocyclic complexes are considered to mimic the synthetic models of metalloporphins and metallocorrins due to their intrinsic structural properties. Macrocyclic complexes have also received special attention because of their mixed soft-hard donor charater and versatile coordination behavior and their pharmacological properties, i.e., toxicity against bacterial growth. The present work is divided into two parts. Firstly, preparation and characterization of some macrocyclic complexes of Co(II), Cd(II) and Zn(II) and secondly, study of their antibacterial activity.

Part-I

In present work some macrocyclic complex compounds of Co(II), Cd(II) and Zn(II) containing a ligand with tetraoxo tetrahydrazin moiety were prepared by template condensation of malonodihydrazide($C_3H_8N_4O_2$) with different aldehydes.

The macrocyclic complexes [Cd($C_8N_8O_4H_{16}$)(ClO_4)$_2$],

[Cd($C_{10}N_8O_4H_{20}$)(ClO_4)$_2$], [Cd($C_{24}N_8O_4H_{28}$)(ClO_4)$_2$] and [Cd($C_{14}N_8O_4H_{24}$)(ClO_4)$_2$] **(1-4)** were prepared by the reaction of malonodihydrazide, ($C_3H_8N_4O_2$) with cadmium(II) perchlorate hexahydrate in presence of formaldehyde, acetaldehyde, cinnamaldehyde and crotonaldehyde respectively in 2:1:2 molar ratio.

The macrocyclic complexes [Co($C_8N_8O_4H_{16}$)(ClO_4)$_2$] and [Co($C_{24}N_8O_4H_{28}$)(ClO_4)$_2$] **(5-6)** were prepared by the reaction of malonodihydrazide($C_3H_8N_4O_2$), with Co(II) perchlorate hexahydrate in the presence of formaldehyde and cinnamaldehyde respectively in 2:1:2 molar ratio.

The macrocyclic complexes **(1-6)** are characterized by elemental analysis, UV-visible and IR spectral analysis, magnetic moment and conductance measurements and some other physical properties.

Part-II

The antibacterial activity of the macrocyclic complexes **(1-6)** and the ligands have been studied against five pathogenic bacteria (viz *Preudomonas areruginosa, Klcbsilla, Shigella flexneri, scherichia coli* and *Bacillus ccreus*) and the minimum inhibitory concentrations of the complex **3** was observed. Among the six complexes, complex **3** is showed medium activity against one pathogenic bacteria(*Bacillus ccreus*). The results were compared with the standard compound, Kanamycine.

Chapter - 1

INTRODUCTION

CHAPTER I

1.1 GENERAL INTRODUCTION

The area of inorganic chemistry which most widely developed in the last few decades is mainly due to coordination chemistry and applies very particularly to the coordination compounds of transition metals. The coordination chemistry is an important and challenging area of modern inorganic chemistry.[1] Recently, it has been an established fact that inorganic chemistry had evolved as a most basic, dynamic and revolutionary science in the field of academics and industrial researches.[2]

Coordination Compounds are the backbone of modern inorganic and bio–inorganic chemistry and chemical industry.[3] Coordination compounds play a very significant role in our lives; the study of them has contributed to the highest degree of understanding the chemical bond in inorganic chemistry. As whole classical coordination chemistry deals with the formation of adducts by metal in their higher oxidation states bonded to inorganic or organic ions or molecules. Interest in both basic and hi-tech research with these materials continues at a rapid pace.

The modern study of coordination compounds begins with two famous men, Alferd Werner and Sophus Mads Joargensen. Although S.M. Joargensen (1837-1914) started the extensive studies on the synthesis of complex compounds, it was not until 1906 when the recognition of the true nature of complexes began with Alferd Werner in 1893, proposed the coordination theory.[4] For this pioneering work Alferd Werner received the Nobel price in 1913. In fact he was the founder of modern coordination chemistry who postulated the first successful theory, known as "Werner's Coordination

Theory", to explain the formation, properties and stereochemistry of coordination compounds. The independent approaches of Sidgwick[5] and Lowry[6], who proposed that a chemical bond required the sharing electron pair led to the idea that a neutral molecule with an electron pair (Lewis –base) can donate these electron to a metal ion or other electron acceptor (Lewis-acid). Although the electron pair donor concept of Lewis[7] is still useful for many Lewis acid base interactions for complex formation, it is apparent that the understanding towards the nature of bonding in metal complexes requires more detailed considerations. The detailed and more modern concepts to explain formation of bonds, the associated bond properties, structures, stabilities and the molecular properties as a whole are more conveniently and successfully considered in terms of modern bonding theories the valence bond theory[8], the crystal field theory[9], the ligand theory[10] and the molecular orbital theory[11].

Metals play a vital role in an immense number of extensively differing biological processes. Some of these processes are quite specific in their metal ion requirements, in that only certain metal ions in specified oxidation states can accomplish the necessary catalytic structural requirement. Metal ion dependent processes are found through out the life science and vary tremendously in their function and complexity.

One of the principal themes of bioinorganic chemistry is the synthesis of metal complexes that have the ability to mimic the functional properties of natural metalloproteins.[12,13] Proteins, some vitamins and enzymes contain metal ions in their structure involving macromolecular ligands. The chemistry of metal complexes with multidentate ligands having delocalized π-orbitals,

such as Schiff bases or porphyrins has recently gained more attention because of their use as models in biological systems.

1.2   THE SCHIF BASE LIGANDS

Schiff-bases are generally regarded as good ligands. The orientation of the lone pair on the nitrogen atom means that it can participate in donation into the appropriate metal ion. This donation, in conjunction with the adjacent oxygen atom that is present in Schiff-base derivatives or the heteroatom in pyridine and thiophene derivatives means that a large array of transition metal coordination complexes can be prepared. Schiff-base ligand formation requires conditions that are not very stringent, necessitating only a dry solvent or a method of removing the water produced in the condensation reaction between an aldehyde/ketone and an amine. The lone pair on the nitrogen atom of the imine moiety provides a suitable donor atom for metal ion complexation. These ligands are particularly adept at binding transition metal ions, with the range of uses for these complexes varying from catalysts to biological mimics.[14,15]

Named after Hugo Schiff, a Schiff-base is a molecule that contains an imine moiety with an alkyl or aryl substituent attached to the imine nitrogen atom. Typical conditions for imine formation require a protic solvent that is sufficiently dry to prevent subsequent hydrolysis of the newly formed imine bond. Generally these condensation reactions proceed smoothly, although some reactants (usually as a result of electronic effects) can require forcing conditions such as heating to reflux in a high boiling solvent to remove the by-product, water.[16] The general consensus of the mechanism of formation of the imine, as shown in Scheme 1.1, is that it requires protonation, or the use of a Lewis acid on the carbonyl oxygen atom to enhance the electrophilicity

of the carbonyl carbon. This protonation step precedes (and facilitates) nucleophilic attack of the primary amine on the carbonyl carbon. A 1,3-H shift follows, which facilitates the elimination of water to give the protonated imine, with subsequent disassociation of this species to give the imine product.
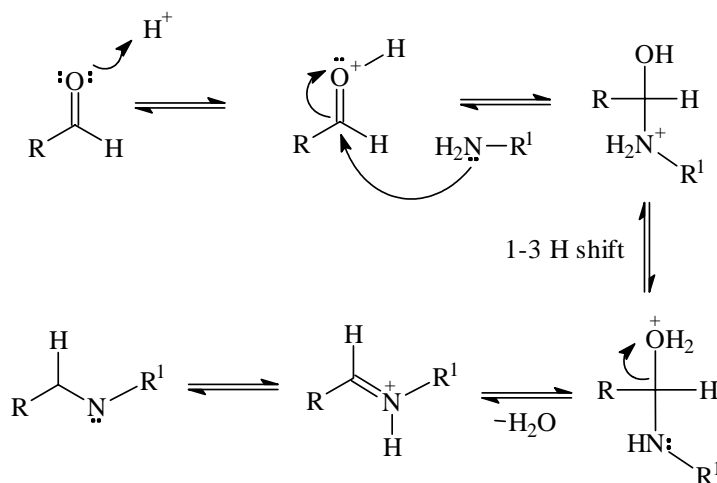
Scheme 1.1: Mechanism of imine formation.

Schiff bases are capable of forming coordinate bonds with many of metal ions through both azomethine group and phenolic group or via its azomethine or phenolic groups.[17-32] A large number of Schiff bases and their complexes are significant interest and attention because of their biological activity including anti-tumor, antibacterial, fungicidal and anti-carcinogenic properties[20-25] and catalytic activity.[25-32]

Naphthylideneimine Schiff base complexes (Figure 1.1) possessing luminescence property, catalyze oxidation of primary and secondary alcohols into their corresponding carbonyl compounds in the presence of N-methylmorpholine-N-oxide (NMO) as the source of oxygen have been reported recently.[27]

Where R= -CH$_3$; Naphmet (HL1)
-C$_6$H$_{11}$; Naphchx (HL2)
-C$_5$H$_4$N ; Naphampy (HL3)
-C$_6$H$_4$Br ; NaphBranil (HL4)
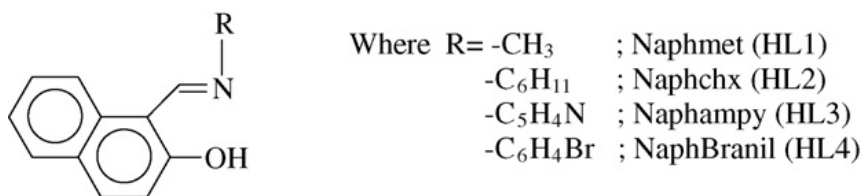
Figure 1.1 Structure of the bidentate Schiff bases.

L-Amino acid Schiff bases with N, O donor system have been reported by Taqui Khan *et al*[33] and are used as catalyst of enantio selective epoxide of 1,2-di hydronaphthalene. Nitro substituted benzaldehyde Schiff bases were used in organic catalytic reactions[34]. Schiff bases of N-methyl and N-acetyl isatin derivatives with different arylamines have been prepared and screened for anti conversant activities[35].

Antibacterial screening of monobasic bidentate Schiff base complexes

(Figure 1.2) with N, O donor have been reported[36].
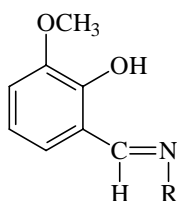
(R = -CH$_3$, -C$_5$H$_4$N, -C$_6$H$_{12}$)

Figure 1.2: Structure of monobasic bidentate Schiff base complexes with N, O donor.

Schiff bases of ethylenediamine/triethylenetetramine (salen) with benzaldehyde/cinnamic aldehyde/salicylaldehyde as corrosion inhibitors of zinc in sulphuric acid have been reported by Desai *et al*[37].

5

Schiff bases have been used as intermediates in the preparation of many organic products. They are used as fungicide and herbicides and appear to be active reagents against the plant enemies. Veil *et al*[38] reported the action of many Schiff bases on the germination of seeds and their transition metal complexes have been successfully used in dye and pigment industry. Thermally stable polymeric metal chelates of Schiff bases may be fabricated easily to form materials of high mechanical strength which are gaining interest. Schiff base complexes have also other momentous physiological properties such as activity, anti-inflammatory activity and analgesic activity. The properties of the complex metal ions are often strongly dependent on the ligand structure. And because of a considerable synthetic flexibility of the complex metal by using suitable designed Schiff base ligands. The ligand and their metal compounds have acquired wide interest in application to biological systems[39] as catalyst. There are many metal compounds which have pharmacological effect and used as active ingredients.

Most of the binuclear complexes have been prepared from tridentate Schiff bases containing ONO or ONS donor atoms; they are derived from the condensation of salicylaldehyde or acetylacetone with o-aminophenols, aminoalcohols, o-aminothiophenols and aminothiols (as in Fig. 1.3).

H₂SAP

H₂ASP

H₂SAE

Fig 1.3

6
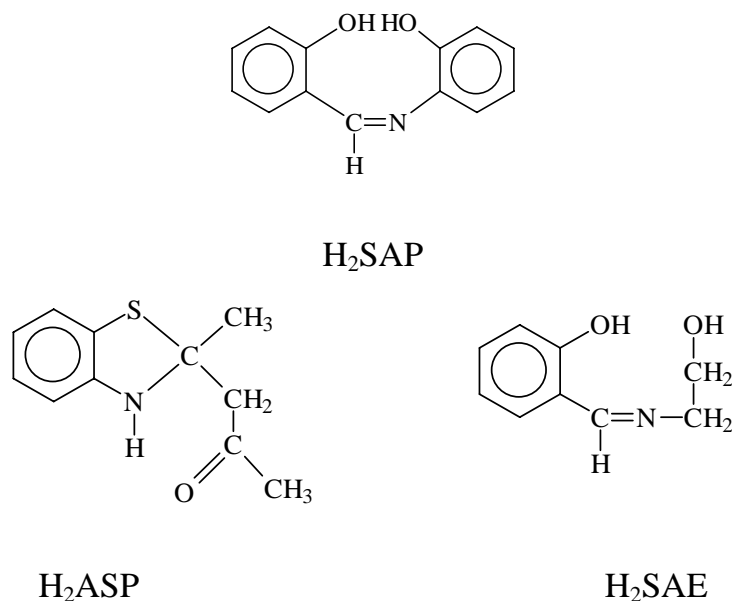
When these ligands react with metal ions, their tridentate character leads to polymerization and polynuclear complexes with anomalous magnetic properties. It was presumed that the copper (II) ions of these chelates would have an unusual co-ordination number of three .[40, 41]

Some examples of open chain Schiff bases: Are given below as in Fig. 1.4
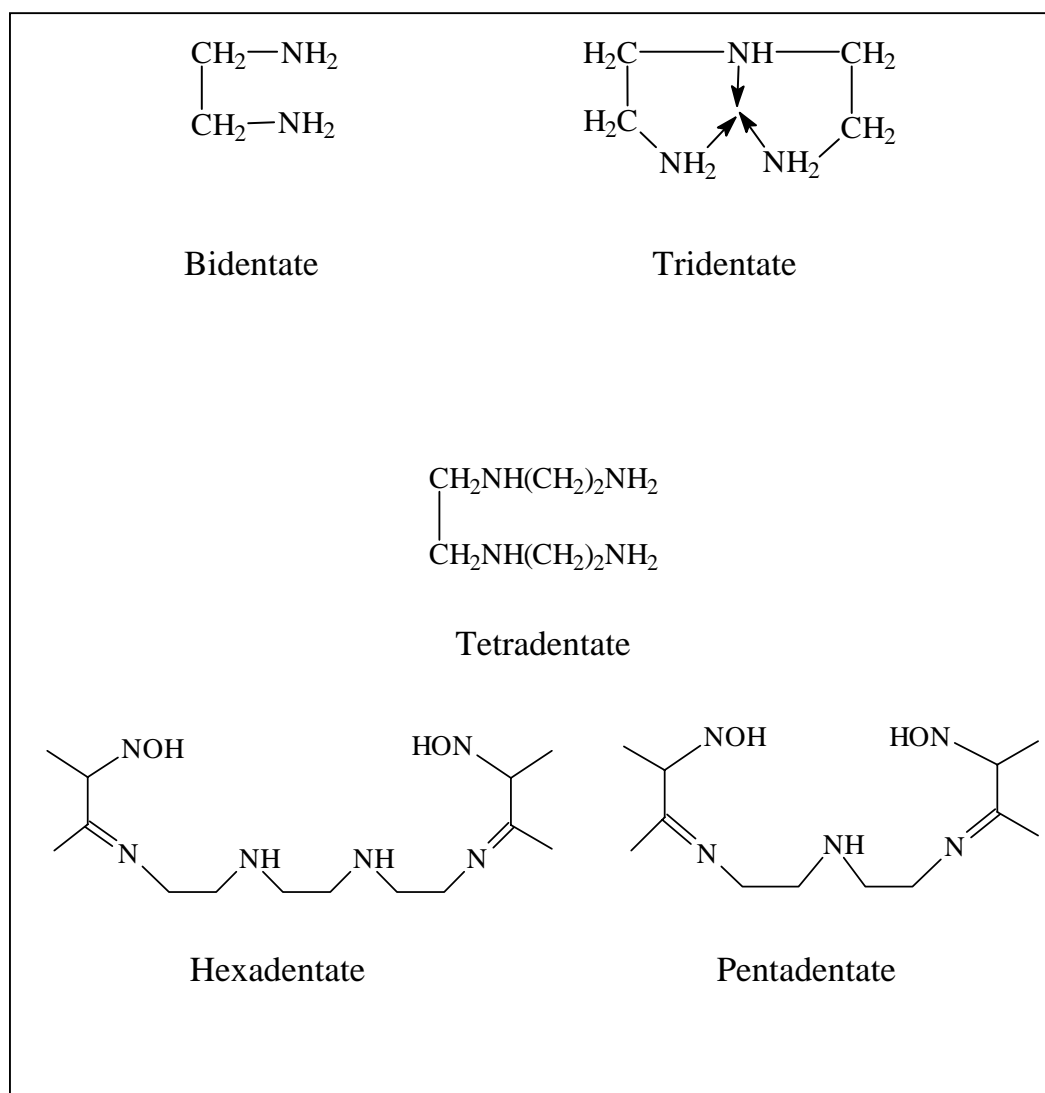
Fig. 1.4

Schiff bases containing polydentate group[42] have not only produced stable metal compounds but also these ligand and their metal compounds play significant role in stereochemistry, structure, isomerism, spectroscopy, kinetics and mechanism of reactions, model system of biochemical interest, stabilizes, polymers, photography, electro optical display devices and agriculture. The chelating properties of these ligands are of interest to many authors.[43]

The commonest donor atoms in the Schiff base ligands are oxygen and nitrogen, less commonly halogen, sulphur, photosphorus, arsenic and carbon atoms act as donors. In some cases bond appears to act as donors. Some examples of such kinds of Schiff bases ligands are depicted below[44] (as in Fig. 1.5).

Nitrogen-sulfur donor ligand

Nitrogen-phosphorous ligand

Fig. 1.5

8
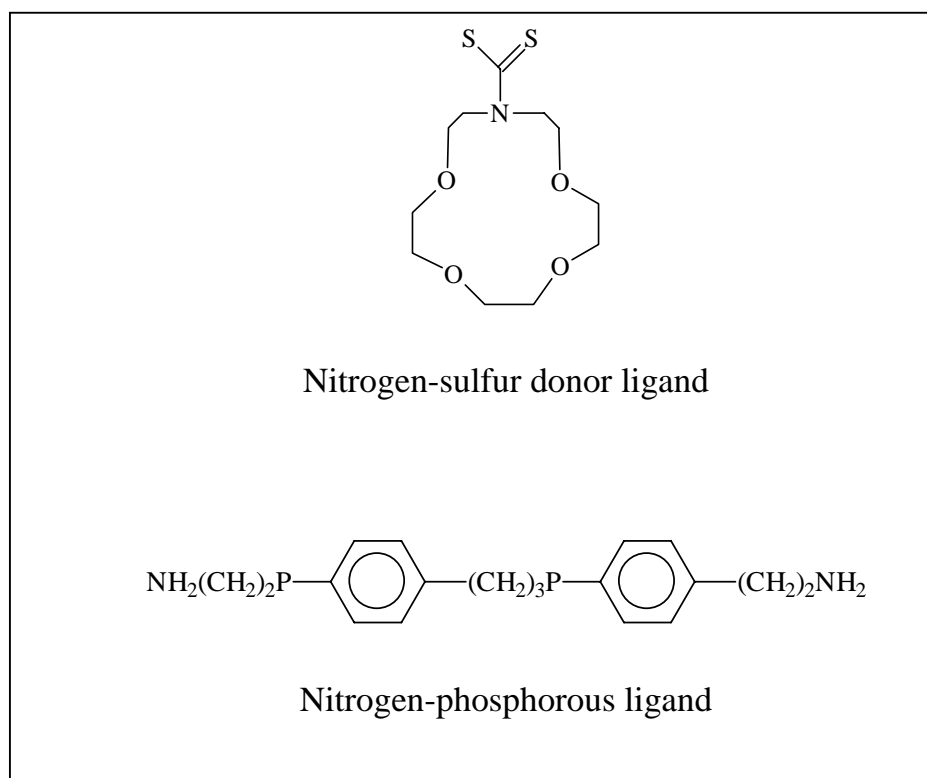
1.3 LIGANDS

In coordination chemistry, a ligand is an ion or molecule that binds to a central metal atom to form a coordination complex. The bonding between metal and ligand generally involves formal donation of one or more of the ligand's electron pairs. The nature of metal-ligand bonding can range from covalent to ionic. Furthermore, the metal-ligand bond order can range from one to three. Ligands are viewed as Lewis bases, although rare cases are known involving Lewis acidic "ligands." There are many kinds of ligand.[45, 46]

1.3.1 CLASSIFICATION OF LIGANDS

Ligands are classified in many ways: their charge, their size (bulk), the identity of the coordinating atom(s), and the number of electrons donated to the metal (denticity or hapticity). Denticity (represented by κ) refers to the number of times a ligand bonds to a metal through non-contiguous donor sites. Many ligands are capable of binding metal ions through multiple sites, usually because the ligands have lone pairs on more than one atom. Ligands that bind via more than one atom are often termed chelating.

1.3.2 MONODENTATE LIGANDS

The ligands which have only one donor atom or are co-ordinated through one electron pair are called monodentate ligands. Such ligands are coordinated to the central metal ion at one site or by one metal- ligand bond only. These ligands may be neutral molecules or in anionic form. Examples are : F^-, Cl^-, Br-, CN^-, NH_3, H_2O, CH_3COO^-, OH^- etc.

1.3.3 BIDENTATE LIGANDS

A bidentate ligand has two points at which it can attach to the central metal atom. Neutral bidentate ligands include the following diamines diphosphines and diethers, all which form five membered rings with a metal atom. Below the Fig. 1.6 & 1.7 as follows:
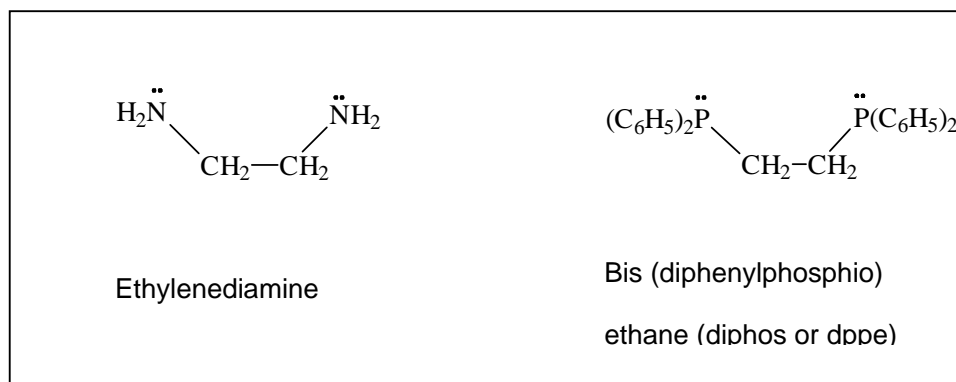
Fig. 1.6
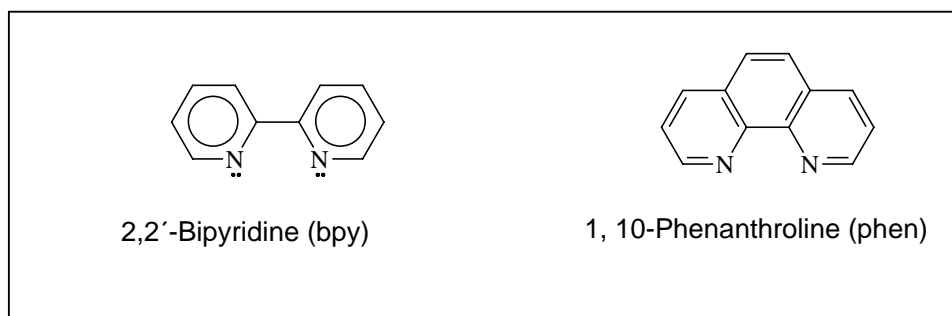
Fig. 1.7

1.3.4 TRIDENTATE LIGANDS:

The tridentate ligands are the molecules which can from three coordinate bonds simultaneously with the same metal ion. Some of the most important tridentate ligands are as follows (Fig.1.8).
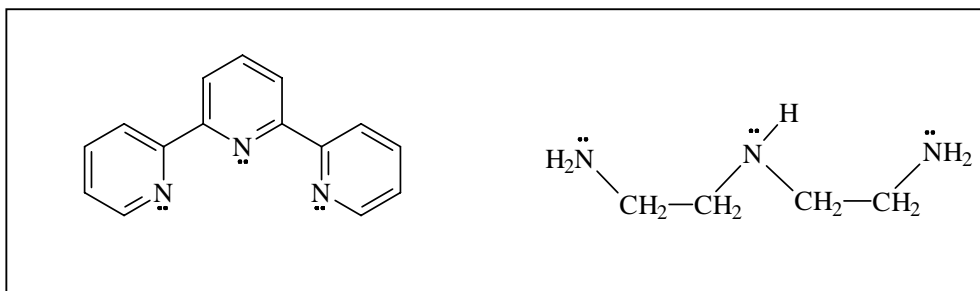
Fig. 1.8

1.3.5   TETRADENTATE LIGANDS:

A tetradentate ligand can be defined as any molecule or ion that has four pair of electron which can be donated to the central meatal ion. Then the molecule or ion is called tetradentate ligand.[47] (as in Fig. 1.9)

Fig. 1.9

11
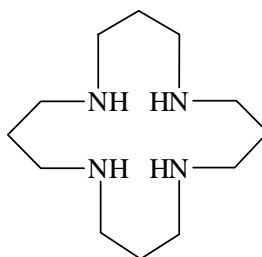
1.3.6 CLASSICAL OR SIMPLE DONOR TYPES

These ligands act as pair donor to acceptor ions or molecules and form complexes with all types of Lewis acids, metal ions or molecules.

1.3.7 NON- CLASSICAL TYPE

Complexes with multidentate ligands in which several donor atoms of the ligand are attched to the same central atoms, producing a cyclic structure, are called chelate complexes.[48] The greatest tendency to form chelate complexes is found in polyfuntional ligands, whose donor atoms are five and six member respectively. Metal chelation is involved in many important biological processes, where the coordination can occur between a verity of metal ions and a wide range of ligands. Many types of ligands are known and the properties of their derived metal chelates have been reported in the literature.[49]

1.4   TRANSITION METAL COMPLEXES

The phenomenon of complex formation is really a very general one, but is especially noted among the transition metal ions. For bonding, the metal must posses vacant orbitals and these orbitals symmetrically must be correct, sterically available and of reasonably low energy. Since transition metal ions generally meet these requirements best, it is not surprising that they form complexes readily.

The transition elements play vital role in coordination complexes mainly because of the following characteristic[50-53]:

- ➢ variable oxidation state (electron transfer properties),
- ➢ coordination geometries (octahedral, tetrahedral, square planar, pyramidal, etc.),
- ➢ spectral and magnetic features, ligand field effects, unpaired d-electrons,formation of chelated complexes,
- ➢ most M^{2+} and higher oxidation states are borderline or hard acids and generally prefer borderline or hard base such as O and N-donor groups; lower oxidation states e.g. Cu(I) are softer acid will bind the soft bases such as O_2, CO, N_2, and S. and
- ➢ formation of polynuclear metal species e.g. dimers, tetramers with bridging.

Metal coordination complexes have a wide diversity of technological and industrial applications ranging from catalysis to anticancer drugs[54].

1.5 THE MACROCYCLIC LIGANDS AND BEHAVIOUR

The large ring compound whose structures are that several donor atom's can bind to a metal most commonly nitrogen donors. However, mixed N,O; N,S; N,O,S; N,O,P; and so on, donors are known. Depending on the donor atoms these can be designated N_4, N_2, O_2, O_4 and so on. The heterocycles can be broadly classed into these without and those with conjugated л system.

1.5.1 BROAD CLASSIFICATION OF MICROCYCLIC LIGANDS: [55]

Planar with unsaturated rings as in porphyrin and its derivatives, the metal atom may be out of the plane of the N donor atom ligand (as in Fig. 1.10).
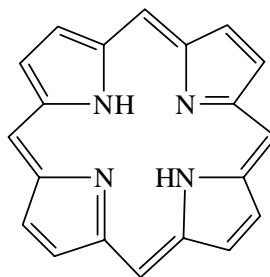
Fig. 1.10

1.5.2 MACROCYCLIC COMPLEXES HAVE THE FOLLOWING CHARACTERISTICS.[56]

  i. A marked kinetic inertness both to the formation of the complexes from the ligand and metal ion.

 ii. They can stabilize high oxidation state, that are not normally readily attainable, such as Cu(III) or Ni(III).

iii. They have high thermodynamic stability than the formation constants for non-macrocyclic N_4, ligand. Thus for Ni(II) the formation constant

for the macrocyclic tetradentate Ni complex (Fig. 1.11) is about five orders of magnitude greater than that for the non-macrocyclic tetradentate Ni complex (Fig. 1.12).
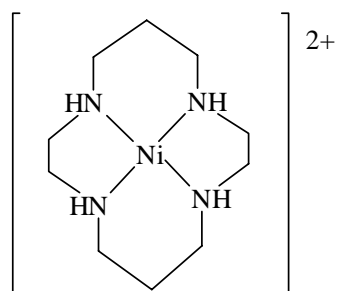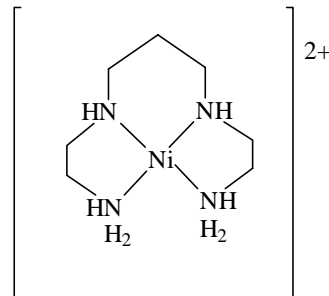
Fig. 1.11   Fig.1.12

1.6   UNSYMMETRICAL SCHIFF BASE LIGAND

An unsymmetrical Schiff base ligand as in Fig. 1.13, that is very resistant to oxidation and consequently useful for complexing metals in high oxidation states.[57]
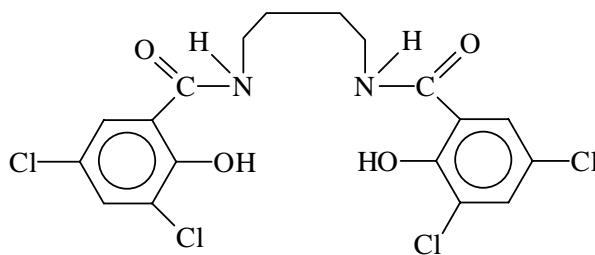
Fig.1.13

1.7 PREPARATION AND MECHANISM OF SCHIFF BASE LIGANDS [58, 59]

The Schiff base ligands are formed by the condensation of primary amine and aldehydes or ketones which looses the acidic protons and behave as chelating agent (as in Fig. 1.14).
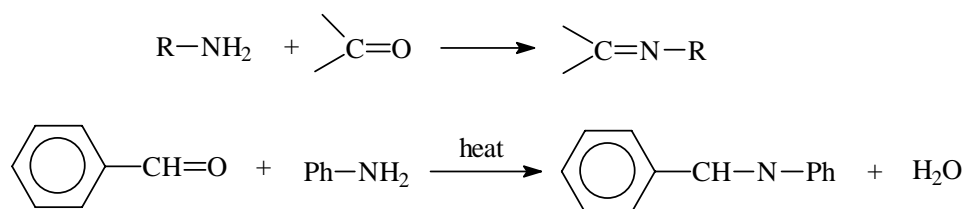
Fig.1.14

Mechanism

The mechanism of imine formation begins as a nucleophilic addition to the carbonyl group. In this case, the nucleophile is the amine, which reacts with the aldehyde or ketone to give an unstable addition product called a carbinolamine. A carbinolamine is a compound with an amine group (-NH$_2$, -NHR or –NR) and a hydroxyl group of the same carbon as in the following reactions given bellow (Fig. 1.15).

Fig. 1.15

16
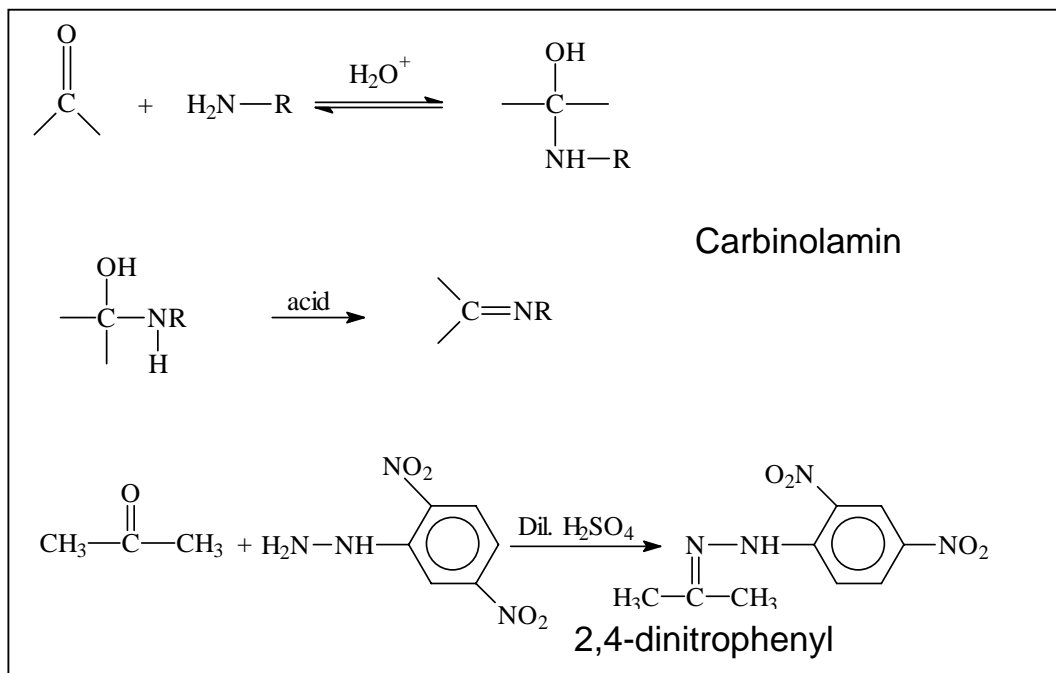
1.8 HYDRAZONE OR CARBOHYDRAZONE SCHIFF BASE LIGANDS AND THEIR METAL COMPLEXES

In the recent years there has been a growing interest in the chemistry of hydrazides, hydrozones or arylhydrazones owing to their pronounced biological activity and analytical applications[60,61,62]. They also form a class of important compounds in medicine and pharmaceutical field. Besides, these ligands have interesting ligational properties due to the presence of several potential coordination sites.[63] They have been widely used as ligand in the preparation of a variety of transition metals complexes and their complexes also showed biological activites and mesogenic properties.[64] The biological activities may be responsible for the azomethine linkage[65,66].

The tuberculostic activity of the arylhyrdzone or their hydrazides have been attributed to the formation of stable chelates with transition metals presents in the cell.[67] The tendency of the arylhydrazone or carbohydrazone moiety to coordinate to the metal through enol form[68] becomes greater as the conjugating ability of the R group in the hydrazine residue increases.[69, 70]

[R(CO)NH–N=C<] ⟷ [R–C(OH)=N–N=C<]
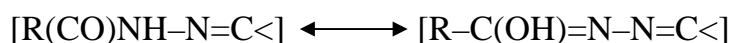
Ibrahim *et al*[71] has prepared some hydrazone ligands by mixing furan-2-carboxyldhyde and benzoic, *p*-methyl or *p*-nitrobenzoicacid hydrazide in absolute ethanol (as in Fig. 1.16).

$$X = H$$
$$X = CH_3$$
$$X = NO_2$$
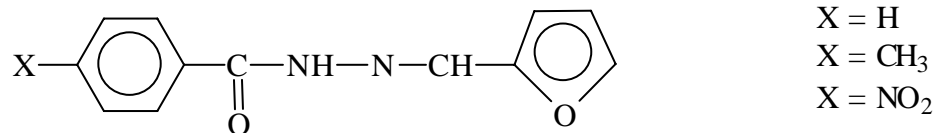
Fig. 1.16

Salicylaldehyde-4-methoxybenzoylhydrazone, [H$_2$SMBHON] (as in Fig. 1.17) and diacetylmonoxime–4–methoxybenzoyl hydrazone, [H$_2$DAMMBHON] (as in Fig. 1.18) reacts with NiX$_2$. nH$_2$O [X= Cl, OAc], to give octahedral[72,73] and square planar nickel(II) complexes which are

[Ni(SMBHON)(H$_2$O)$_3$], and [Ni(DAMMBHON)H$_2$O] respectively.

H$_2$SMBHON
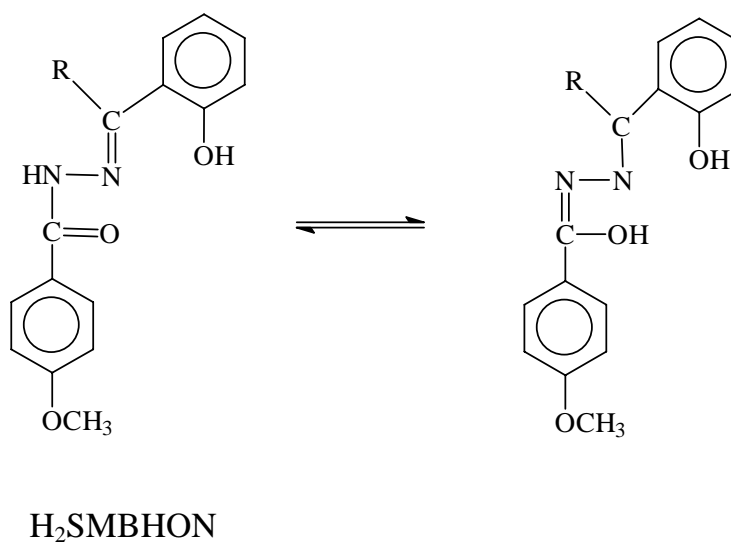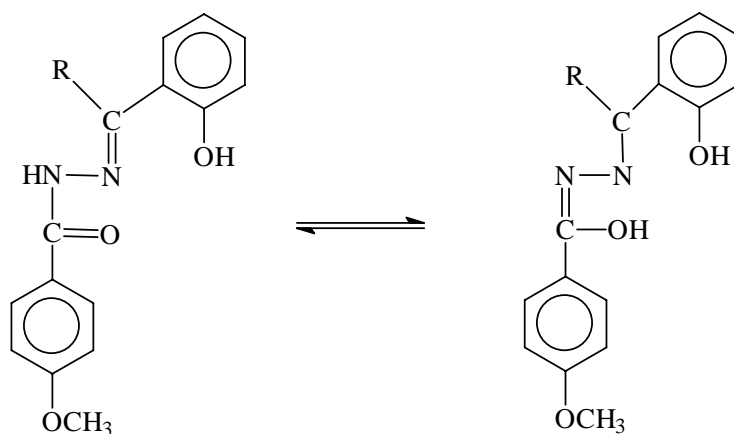
Fig. 1.17

H$_2$DAMMBHOM

Fig. 1.18

Metal complexes of aroylhydrazones have broad application in biological process such as in the treatment of tumour[74,75] tuberculosis, leprosy[76] and mental disorders. These are also known to act as herbascides[77], insecticides and herbicides[78,79]. The biological activity has been attributed[80] to the complex forming abilities of ligands with the metal ions present in the cells. These ligands can act both as neutral and mononegative and favour certain geometries[81,82] to the complexes. Several new metal complexes of Co(II), Ni(II), Cu(II), Zn(II), Cd(II), and Hg(II) with hydrazones derived from benzoic, *p*-methyl or *p*-nitrosubstituted acid hydrazide and furan-2-carboxyldehyde which are reported.[83,84]

Jahagirdar *et al*[85] have synthesized complexes of malonoanilic acid, hydrazones with Co (II), Ni(II). In this case he pointed out that the hydrazone ligands behave in a dibasic tetradentate manner in all the complexes except in the case of Cu (II) complexes, where they act as dibasic tridentate ligands[86]

with O:N:O donor sequence. The acylmonohydrazones, (as in Fig. 1.19) have synthesized by condensing the hydrazide and the corresponding aldehyde in ethanol.

R = H
R = CH$_3$
R = Cl
R = OCH$_3$
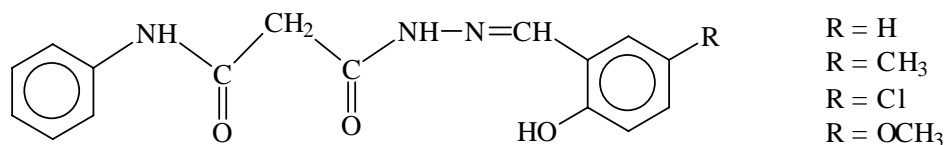
Fig. 1.19

Dutta *et al*[87] prepared the Shiff baseligands, 2-benzoyl pyridine anthraniloyl hydrazone and bis(2-benzoyl pyridine)benzyl hydrazone (as in Fig. 1.20 and 1.21) by the condensation of anthraniloylhydrazine and benzildihydrazine respectively with 2-benzoyl pyridine and their compounds of Co (II), Ni(II) and Mn(II).[88, 89]
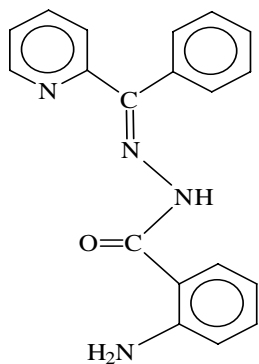
Fig.1.20
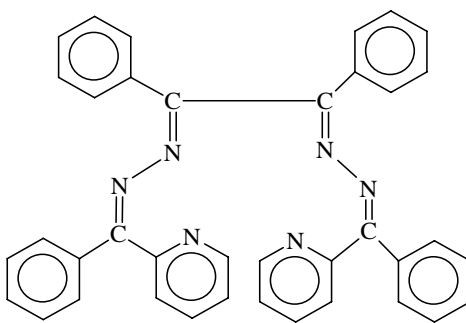
Fig. 1.21

1,4- Dihydrazinophthalazine and its hydrazone with various aldehydes and ketones[90] have been tested systematically for their anti-bacterial and

20

antitumer. Okur *et al*[91] has synthesized 1,4-bis (α- carboxybenzyl idenehysrazino) phthaline (as in Fig.1.22). Rao *et al*[92, 93] have been synthesized various hydrazides and their Schiff bases[94, 95] by the reaction of 2-acetylpyridine isonicotinyl hydrazines with aldehydes, one of them is 2-acetylpyridine isonicotinyl hydrazone[96] (as in Fig. 1.23).
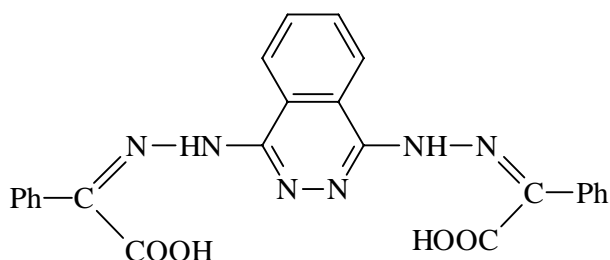
1,4 bis(α–carboxybenzyli denehydrazino) phthaline

Fig. 1.22
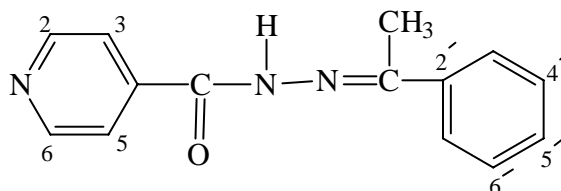
Fig. 1.23

1.9 TEMPLATE SYNTHESIS OF MACROCYCLIC COMPLEXES

Macrocycles that are saturated or have double bond in only one part of ring can be made independently or can be made by template synthesis[97], where the presence of a metal ion controls the ligand synthesis. The term "template synthesis" has been suggested for reactions in which metal ion coordinate ligands which then react to form chelate rings. In short these are reactions in which the presence of the metal ion controls the synthesis. [98, 99,100]

A great variety of nitrogen containing macrocycles can be synthesized by using metal ion as a template. Some representative reactions are as follows (as in Fig. 1.24):
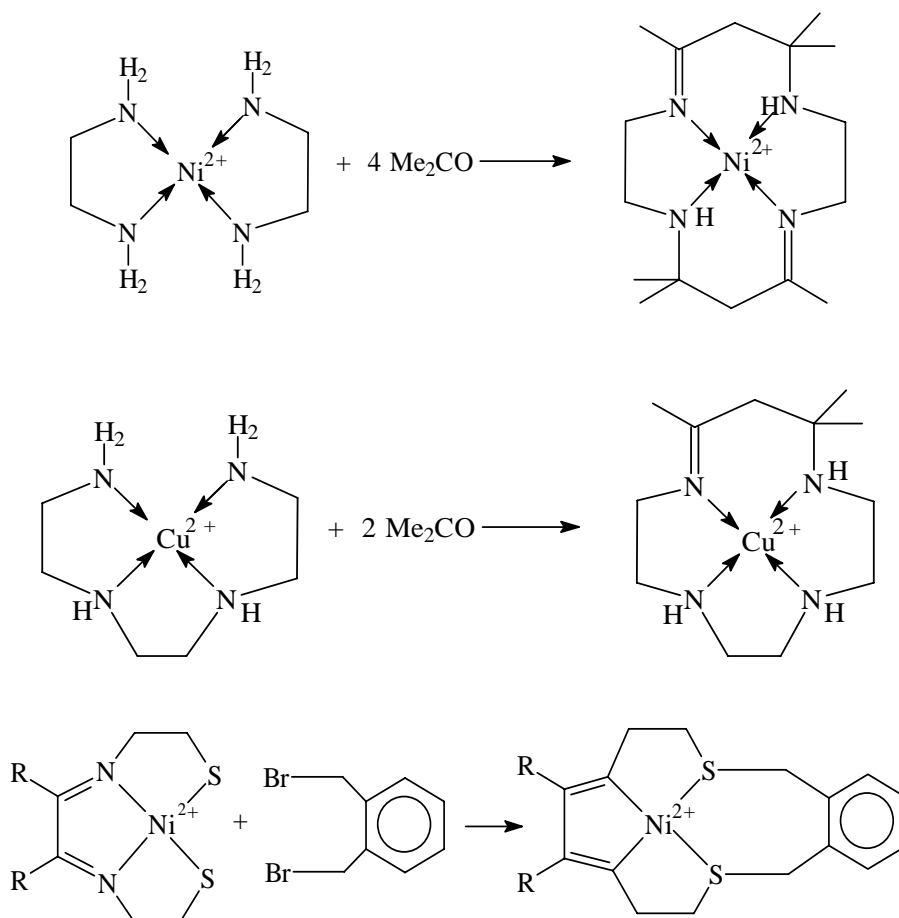
Fig. 1.24

The template synthesis of macrocyclic ligands is one of the best examples of metal ions affecting the sterric course of a reaction. Unsaturated poly azomacrocycles and their metal complexes, have received special attention since they are considered as useful models for biological macrocyclic systems. Consequently, metal template cyclization reactions of 1,2-dihydrazones have been extensively studied for this purpose. 1,4-

Dihydrazinophthalazine and its hydrazones with various aldehydes and ketones, have been tested systematically for their anti-bacterial and antitumor activity.[101]

A great variety of nitrogen containing macrocycles can be made by employing the Schiff base condensation reaction often with a metal ion as template. A representative reaction is as follows:[102] (as in Fig. 1.25)
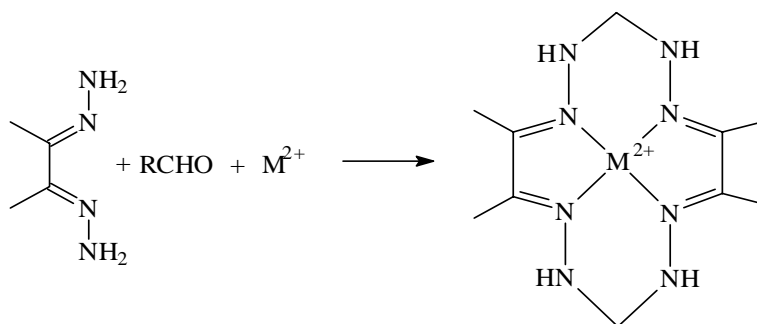
Fig. 1.25

The diacetyl dihydrazone reacts with Ni(II) in the presence of formaldehyde in different ratio to give the octaazamacrocyclic(14-membered) macrocyclic ring (as in fig. 1.26).
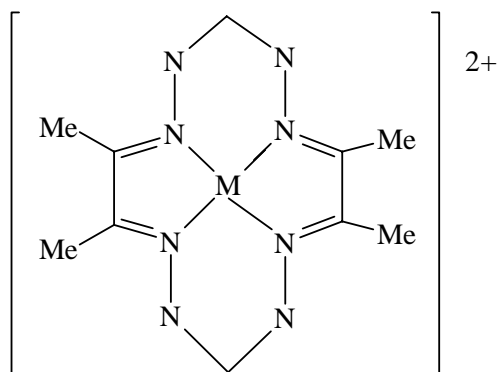
Fig. 1.26: Template complexes formed by diacetylmonoxime hydrozone and formaldehyde.

It is to be noted that there must be some control of the reaction by the size of the metal ion used. If the ion is too small or too large, no macrocyclic complex may be formed.[104,105]

1.10 MACROCYCLIC COMPOUNDS CONTAINING "OXO" GROUP

In the literature[106,107,108] it is reported that a 16-membered tetrapeptide macrocycle (as in Fig. 1.27) has been obtained from the reaction of 1,2-diaminoehane and phthalic anhydride in dioxane which was characterized by physicochemical methods.[109,110]

R. W. Hay and P. R. Norman prepared[111,112,113] the 14-membered macrocyclic diamide-5,7-dioxo-1,4,8-tetraazacyclotetra decane from 1,9-diamino-3,7-diazanonane and diehyl malonate (as in Fig. 1.27, 1.28).
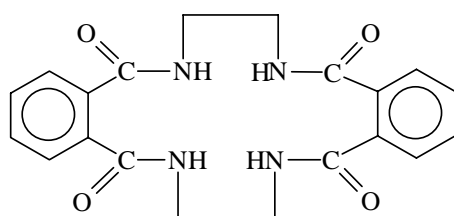
Dibenzo [e,k][1,6,9,14] Tetra

Azacyclohexadecane[2,5,10,13] Tetraone

Fig. 1.27
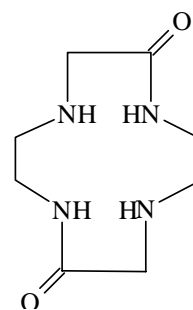
1,4,7,10-Tetra-azacyclo-

dodecane-2,6 dione

Fig. 1.28

The preparation of a number of cyclic 14-member dioxotetraamines was first described by Tabushi *et al*[114] the 13- and 15-membered ring compounds have also been prepare[115,116,117] and the equilibria and kinetics of copper(II)

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complex formation were studied. Hill and Raspin[118] first reported the preparation of copper(II), nickel(II) and cobalt(II) complexes of the linear dioxotetraamine. It is of considerable interest to macrocyclic amides ligands of this general type bear a structural resemblance to tripeptides as gly-gly[119] which are well known to ionise two amide protons on coordination to metal ions such copper(II)[120]. The gly-gly ligand is currently used for the treatment of Wilson's disease.

Macrocyclic diamides may also be capable of stabilizing copper(III)[121,122] and nickel(III) [123,124] as occurs with a number of peptide ligands. Stable nickel(III) complexes of a number of saturated and unsaturated macrocyclic tetra-aza ligands have also recently been described.[125]
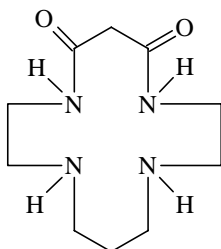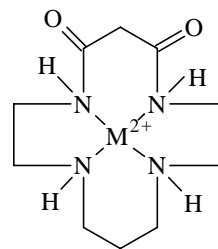
Fig. 1.29   Fig. 1.30

Co(II) complexes of porphyrins, Schiff bases and tetraaza systems (Fig. 1.31(A-C) are usually studied as models for oxygen carriers. Schiff bases used for the studies of oxygen carrying properties are generally tetradentate, of which at least two of the donor atoms should be nitrogen, with the others being nitrogen, oxygen, sulphur (or) combination of the three.

(A)
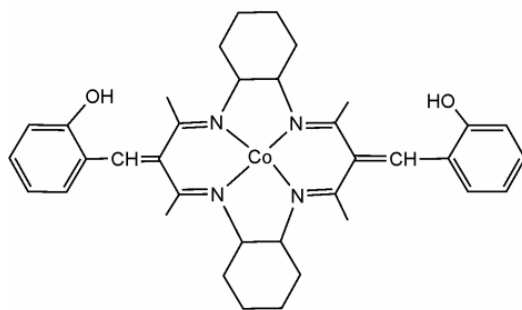
(B)
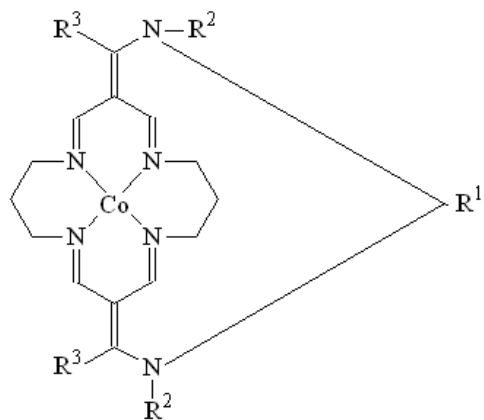
(C)
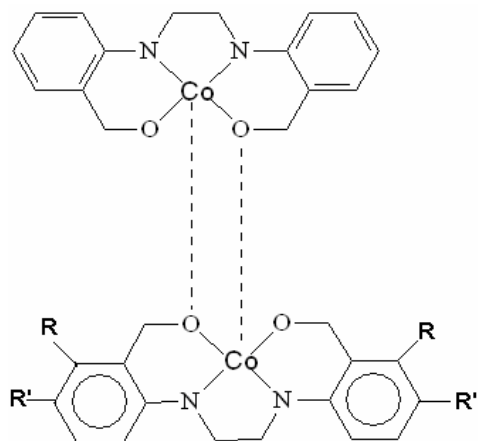
Figure 1.31: (A-C) Co(II) complexes of porphyrins, Schiff bases and tetraaza systems.

1.11 COORDINATION COMPLEX OF PERCHLORATE ION

Generally transition metal perchlorate salts form complex compounds of ionic spices. This is because the negative charge on perchlorate moiety stabilized by resonance structure, hence the prefer to form ionic spices still there are some neutral transition metal complexes having perchlorate moiety, where the perchlorate moiety is in coordinate state with the metal.

Twenty member N_4 binucleating copper(II) macrocyclic complexes of L^1, L^2, L^3 have been derived by a cyclic (2+2) condensation of diformylthiophene or diformylfuran with the appropriate diamine. It is reported in the literature that the complexes $[Cu_2(L^1)(OH_2)_2(ClO_4)_2$ $]H_2O$ and $[Cu_2(L^3)(OH_2)_2(ClO_4)_2]$, which are the coordinated to the metal perchlorate. The complexes showed IR band[126] at (1085-620) cm^{-1}. The macrocyclic ligand denoted by L^1 and L^2 are given as follows.(Fig. 1.32)
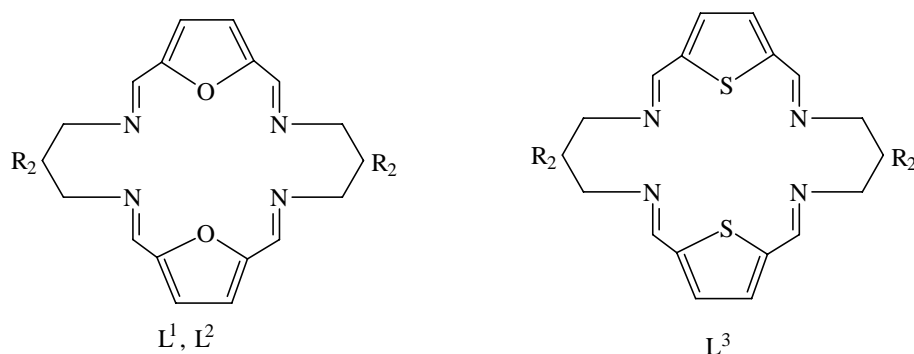
When R = H, L^1 and L^3; R = CH$_3$, L^2

Fig.1.32

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Reaction of $L_n(ClO_4)_3$ with SMMT give the $L_n(SMMT)_2ClO_4]$ complex (where $L_n = L_a$, Ce, Pr. Nd. Sm, Eu, Gd, Tb, Dy and Y) and [SMMT = 4-Salicylideneamino-3-mercapto-6-methyl-1,2,3,4-triazine(4H)-5-one]. [127]

The above complexes showed the conductance values (12.25 – 10.44) ohm^{-1}cm^2 mol^{-1} which is non-electrolytes.[128] The complexes showed IR bands at 1150, 1080, 660 cm^{-1} for the vibration v_1, v_2, v_3 respectively. [129] (as in Fig. 1.32)
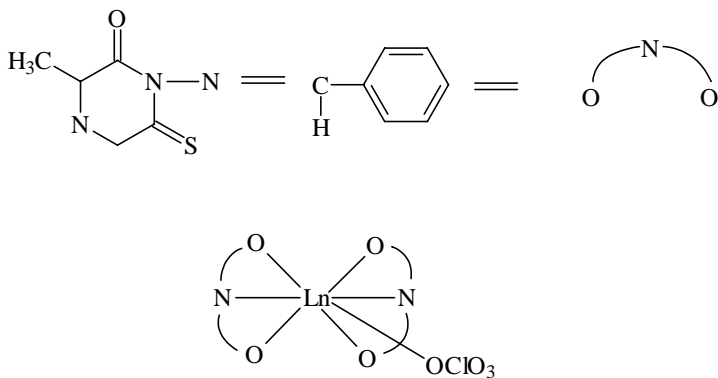
Fig. 1.33

Synthesis of dinuclear copper(II) complexes of tetraimine Schiff-base macrocyclic ligand derived from 2,6–diacetyl pyridine and 1, n-diamino-n′-hydroxyalkanes (nn′=3,2,4,2 and 5,3) complex $[Cu_2(HL^4)(H_2O)_2(CLO_4)_3]$ and $[Cu_2(H_2O)(ClO_4)_3]$ are indicated the coordination of perchlorate to the metal[130] as shown Fig. 1.34:

H_2L^4; R = $CH_2CH_2CH(OH)CH_2CH_2$
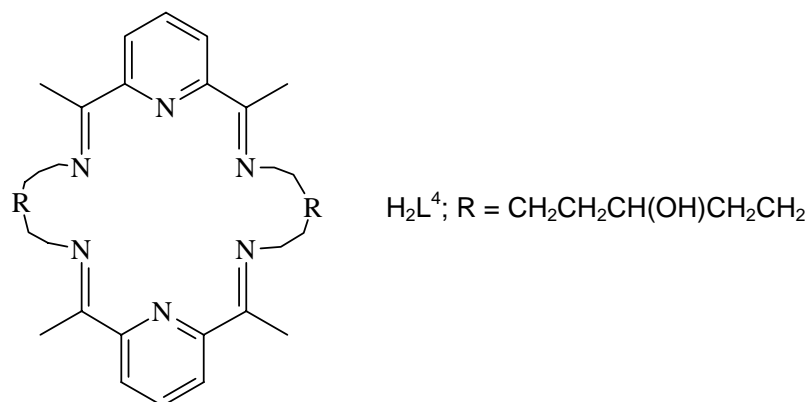
Fig. 1.34

Lead(II) complexes are prepared by template cyclizations in the tetrahydrobate reduction of the Schiff base.[131] The perchlorate coordinated complexes [Pb(L^8)(ClO_4)$_3$EtOH.H_2O] are given as follows (as in Fig. 1.35).
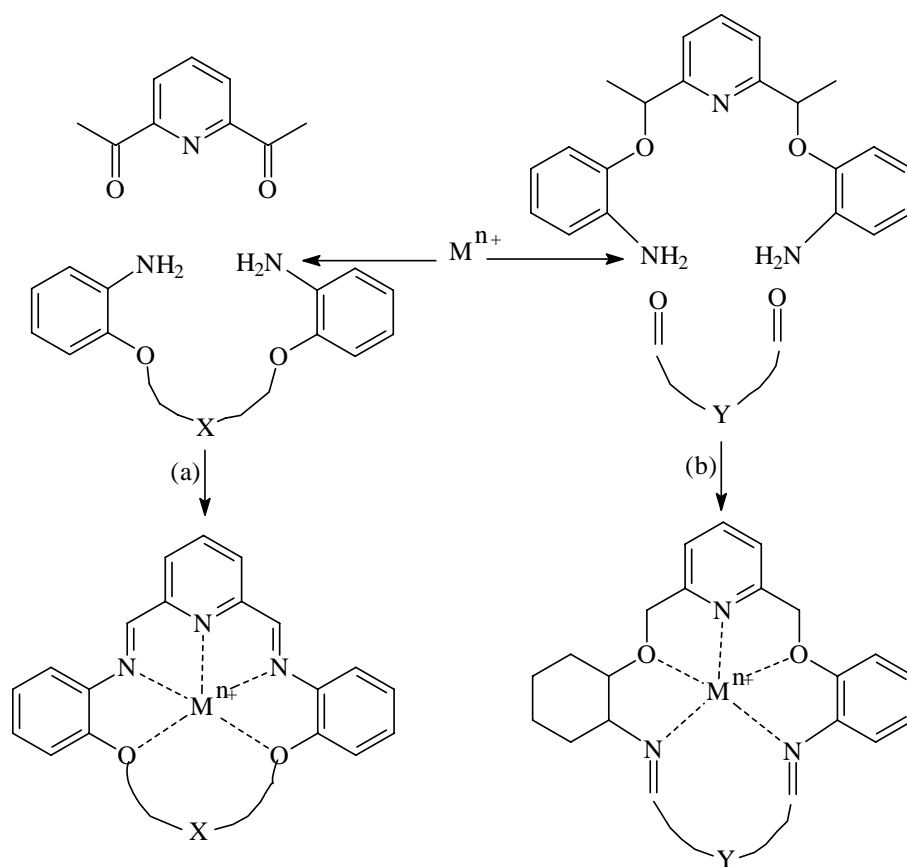
Fig. 1.35

Ligand	M²⁺	X	Ring size	Ligand	M²⁺	Y	Ring size
L⁷							
	Mn		15	L⁵	Pb		18
				L⁶	Pb		18

The above complex coordinate the perchlorate to the metal, because of the suggest IR band at (1098-625) cm^{-1}.

In the literature, it is reported that bis [2,6-bis(1-methylbenzimindazol-2-yl)pyridine]copper(II)diperchlorate monodyhdrate and acetonitrile [2,6-bis(benzimidazo)-2-yl)pyridine] (perchlorate) copper(II)perchlorate[132] complexes coordinate the perchlorate to the metal.

It is also reported that [Ba($C_{34}H_{32}O_4N_4$)(ClO_4)$_2$] and [Sr($C_{36}H_{35}O_5N_4$)(ClO_4)$_2$] complexes coordinate perchlorate to the metal.[133]

Again, in the same phenomenon, it is reported that [NiL^9(ClO_4)] and [CuL^9(ClO_4)] complexes are good agreement of coordination perchlorate bond to the metal. (Where L^9 = 1,8–dibenzoyl-2,7-diphenyl-3,6-diazooctane). The complexes showed absorption bond at (1100, 1080, 980) cm^{-1} for ClO_4 moeity.[134]

In the above arena, it is reported that [Cu$_2$L^{10}(ClO_4) $_2$] complexes are good agreement of coordination perchlorate bond to the metal. This coordination is based on the elemental analysis and electronic spectra and magnetic moment.[135] [Where L^{10} = Bis-{1- hydroxyiminato-1,2-diphenyl(2′-iminopyrid)}ethane].

The complex [Ag(INHSAL)$_2$(ClO_4)] and [Ag(INHHAP)$_2$(ClO_4)] have been synthesised[136] by adding ligand dropwise and mixed with metal perchlorate solution in the 2:1 molar ratio, (where ligand INHSAL = 2-hydroxybenzaldehyde) INHHAP = 2-hydroxy acetophenone. On the basis of IR spectra, it showed a band at (1090-620) cm^{-1} regions. These are assignable that perchlorate coordinate to the metal.[137,138]

1.12 BIOLOGICAL ACTIVITY OF SOME IMPORTANT COMPOUNDS

It is now appreciated that metal ions control a vast range of processes in biology. Many new and exciting developments in the field of biochemistry create interest out of inorganic chemists to court in the new area called "Bioinorganic Chemistry". Complex compounds are 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.[139] The transition metals especially first row transition metal ions are well known for their ability to form wide range of coordination complexes in which octahedral, tetrahedral, and square planar geometries predominate. Some 25 elements which are currently thought to be essential to life, ten can be classified as trace metal ions Fe, Cu, Zn, Mn, Co, Cr, Sn,V and Ni are four as bulk metal ions, Na, K, Mg and Ca. In addition there is some tentative evidence that Cd and Pd may be required at very low levels.

In the following section as outline of the chemistry and biological effects of some of the essential and polluting elements is given. 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 hemocyanins, contain Cu and bind one molecule of O_2 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.[140]

In sufficient levels of cobalt in the diet of ruminants gives rise to wasting disease, known in Australia and Newzealand as bush sickness. Trace amounts of vitamin B_{12} is essential for the synthesis of hemoglobin by mammals; importation of cobalt powder has been reported to cause malignant tumors in muscles.

Metal complexes occupy an important role in almost all branches of medical and pharmaceutical activity. Prior to 1980, search for anticancer drug was focused primarily on organic compounds.[141] However, with the discovery of cis-diaminechloroplatinium(II), which shows excellent antitumour activity, Keen interest arose in exploring other inorganic compounds as possible therapeutic agents, copper, silver and gold complexes are among the most promising inorganic compounds known to posses anticancer activity.

Copper is found in human cells and in enzymes, that are required for normal metabolic process. The complexation of Co, Fe, Mg, Zn and Cu with nitrogen containing chain in the enzymes are very diverse. [142,143] The antimalarials activities of a series of 2-acetyl pyridine and their Cu, Ni, Fe, Mn complexes have tested for their antimalarial and antiteukemie properties. These compounds have been to posses' significant antimalarial activities.[144] Brada and Altaman found copper containing compounds to be effective in preventing liver tumours. Iron(II) complex being much more active. The antineoplastic antibiotics bleomycin has shown to be more effective as it copper complex Rao *et al*[145] investigated antitumour activity and cyctotoxicity of blemocycin with some of it metal complex using Ehrlich ascites tumours in ince and Ehrich cell in culture. They reported the following order for both antitumour activity and cytotoxicity.

$$Cu(II) \ Blm > Zn(II) \ Blm > Fe(II) \ Blm > Co(II) \ Blm$$
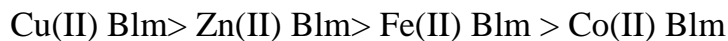
[Blm = Bleomycin]

Although ions of cobalt, copper, iron, zinc and manganese are essential for mammals, but the above metals are in excess, these may cause Wilson disease. Again if excessive accumulation of copper is observed in the liver, kidney and brain, it may create disorder in liver and kidney; and it may also lead to various neurological abnormalities. The disease involves the administration of various cheating agents which are capable of mobilizing the copper as follows:
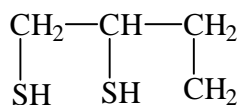
Dimercaarol (BAL)
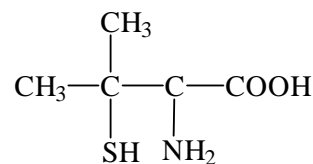
D–Penicillamine
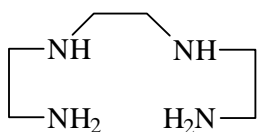
1,8 Diamino-16-diazoctane
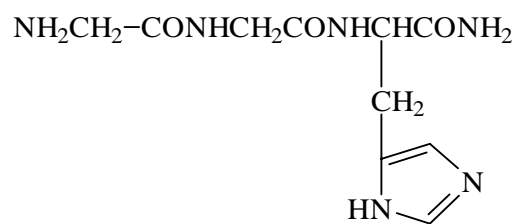
Glyglyhin NH_2

Cancer is a disease characterized by uncontrolled multiplication and spread within the organism of apparently abnormal forms of organism itself. Many organic caseinogens are also excellent ligand. (Fig.1.33) and the importance of coordination compounds, in cancer initiation is the subject of considerable debate.
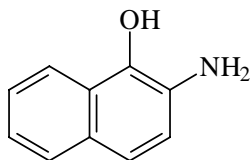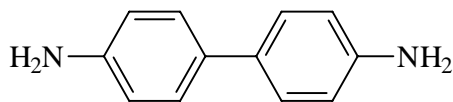
2-amino-l-naphthol   Benzidine

1.13 AIM OF THE PRESENT WORK

The macrocyclic complexes have attracted the attention of both inorganic and bioinorganic chemists in recent years[146]. Naturally occuring macrocycles were shown to be capable of activity transport metal ion across membranes, beginning with valiomycin[147]. Synthetic macrocyclic ligands have been shown to form very stable complexes with alkali and alkaline earth metal cations. These complexes can be used as models for investigation of ion transport throughout membrane in biological systems[148]. The potentialities of tetraaza synthetic macrocyclic complexes as models for more complex natural system is now well recognised[149]. In these macrocyclic complexes, both the metal ion and the size of the ring play an important role. The saturated macrocycles with various numbers of their ring membered have been synthesized consistently. These compounds have produced interesting informations concerning both the stabilities and structure of their metal complexes[150]. It has also been suggested that these are suitable for the treatment of various allergies, asthma and influenza[151]. Several organolead compounds find uses as good algicides, herbicides and also as anticancerous agents[152-154]. The applications[155-157] of macrocyclic compounds in bioinorganic chemistry, catalysis, extraction of metal ions from solution and the activation of small molecules gave impetus to this endeavour.

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In the nature a lot of macrocyclic compounds are known e.g. hemoglobin, myglobin, vitamin B_{12} coenzymes where the metal atoms are Fe, Co,and the basic macrocyclic unit is prophyn but have different functional group to the macrocyclies. The chemical properties of some macrocycliccompounds resemble those of antibiotics.[158] For example, macrolactones resemble the natural metabolites such as nonatine and monactine.

In the recent years considerable attention have been given to the synthesis of macrocyclic complex.[159, 160, 161] These complex compounds have been used a model system of biologically important materials, such as porphyrin and corins.

Some of the macrocyclic ligands cannot be easily prepared from the reactants.[162] In that case the complex compounds could be synthesized by template method. The desire macrocyclic ligand can be isolated by the complex compounds.[163, 164] Macrocyclic complex of Ni^{2+} act as catalyst to reduce CO_2 to CO and Mn^{2+} porphyrins have been most commonly studied catalyst.[165]

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 Co(II), Zn(II) and Cd(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 biological system 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 Co(II), Zn(II) and Cd(II) perchlorate in presence of formaldehyde, acetaldehyde, cinnamaldehyde and crotonaldehyde. The synthesized macrocyclic complexes will be characterized by elemental analysis, UV visible and IR spectra analysis, magnetic moment and conductance measurements and some other physical properties.

ii.   Secondly, study of antibacterial activity of the synthesized complexes (some test organism such as, *Preudomonas areruginosa, Klcbsilla, Shigella flexneri, Escherichia coli, Bacillus ccreus*) including the investigation of minimum inhibitory concentration of the complexes.

Chapter - 2

METHOD & MATERIALS

METHOD AND MATERIALS

2.1 PHYSICAL MEASUREMENTS

2.1.1 Weighing

The weighing operation was performed on an Electronic balance.

2.1.2 Melting Point Measurement

Melting point of the ligands and complexes were obtained with an electrothermal melting point apparatus [Biocote, Model No. SMP 10]

2.1.3 Infrared spectra

Infrared spectra were recorded as KBr with a SHIMADZU FTIR-8400 infrared spectrophotometer.

2.1.4 Conductivity

The conductivity cell was normally cleaned three times with water and finally rinsed three times with acetones and allowed to dry in air.

Conductivity measurement of the present complexes were carried out in dimethyl sulfoxide (DMSO). The conductivity viz. the molar conductivities were calculated by using the formula. $\Lambda = (1000/C) \times$ cell constant $\times$ observed conductivity. Where 'C' represents the concentration of the respective complex in mol/L.

Generally 10^{-3}M solutions of the complex were employed for this purpose. The conductance measurements were made at room temperature by using a digital conductivity meter and a dip type cell with polarized electrons. The cell was calibrated with 0.01N, 0.001N and 0.0001N potassium chloride solution and it has a cell constant of 1.065cm^{-1}. The conductance of the pure solvent was also determined. The observed conductivity was obtained by subtracting the conductance of the pure solvent from the observed conductance of the solution of the complexes.

2.1.5 Magnetic Moments

i.   Working principle of the balance:

The SHERWOOD SCIENTIFIC Magnetic Susceptibility Balance (MSB) is the result of collaboration with professor D.F. Evans of imperial college London and is designed as a replacement for a traditional Gouy balance system the evans method uses the same configuration as the Gouy method but instead of measuring the force which a magnet exerts on the sample, the equal and opposite force which the sample exerts on a suspended permanent magnet is observed.

The M.S.B. works on the basic of a stationary sample and moving magnets. The pairs of magnets are placed at opposite ends of a beam so placing the

system in balance. Introduction of the sample between the poles of one pair of magnets produces a deflection of the beam, which is registered by means of phototransistor. A current is made to pass through a coil mounted between the poles of the other pair of magnets, producing a force restoring the system to balance. At the position of equilibrium, the current passed through the coil is proportional to the force exerted by the sample and can be measured as a voltage drop.

The following general expression for mass susceptibility χ_g in C.G.S. units may be derived in the same manner for the traditional Gouy Method.

$\chi_g = l/m[C(R-R_o) + \chi_{vair}A]$………………………………………(i)

Where,

C = Constant of probability

R = Susceptibility of the tube with sample

R_o= Susceptibility of the empty tube

l = Length of the sample (in cm)

m = Mass of the sample (in g)

A = Cross-section area of the tube (cm^2)

χ_{vair} = Volume susceptibility of the displaced air, for powdered sample the air correction term χ_{vair} may normally be ignored. C, the constant of proportionality is related to the calibration constant of a given balance by the formula.

$C = C_{Bal}/10^9$ ……………………………………………….(ii)

From (i) and (ii), we get

$$\chi_g = C_{Bal} \times 1 \times (R- R_o)/10^9 \times m \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.(iii)$$

ii. Calibration of the balance

The magnetic susceptibility Balance (M.S.B.) must be calibrated at its intended work place. The balance is to be used mainly for solid sample, then a solid celebrant (preferably) [HgCo(SCN)$_4$] is recommended since some of the systemic errors in packing cancel. The constancy of the calibration was checked using a sealed off sample of MnCl$_2$ solution.

iii. Procedure

1. The zero knob of the magnetic susceptibility was turned until numerical display shows zero (000) and calibration sample [HgCo(SCN)$_4$] was inserted into sample holder. It then allowed settle reading the numerical display.

2. Reading was recorded and calibration constant was calculated from the formula.

$C = Blac = C_{Tube}/(R-R_o)$

$\quad = (1766.842)/ \{2830-17\}$

$\quad = 2.086 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. (iv)$

From (iii) and (ii) we get,

$\chi_g = 2.086 \times 1 \times (R-R_o)/10^9 \times m.. \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..(v)$

iv. Operation of the "Balance"

1. The range knob was turned to the XI scale was allowed to 10 minutes warm up period before use.

2. The zero knob adjusted until the display reads 000. The zero was adjusted on each side.

3. An empty tube of known weight was place into the tube guide and was taken the reading R_o.

4. The sample was packed and noted the sample, mass (m) in grams and the sample length (l) in cm.

5. The packed sample tube was placed the tube guide and was taken the reading, R.

The mass susceptibility, χ_g is calculated using.

$$\chi_g = 2.086 \text{ x } 1 \text{ x } (R-R_o)/ 10^9 \text{ x m.}$$

The temperature was read from thermometer situated in the balance room.

v. The Magnetic moment

From the measurement of magnetic moment one can find the number of unpaired electrons present in the system and possible configuration and also the structure.

If substance is placed in a field of intensity H gauss, the magnetic induction of the field within the substance is given by,

$$B = H + 4\pi I$$

Where,

I = Intensity of magnetism induced by the field.

I/H = is called the volume susceptibility of the substance, and is given the symbol χ_v . In most cases, a more useful quantity is the magnetic susceptibility per unit mass susceptibility, χ_g equal to $\chi_{v/d}$ where d is the density of the substance in g/cm^3. It is convenient to regard χ_v as dimensionless and χ_g as having the dimensions of reciprocal density.

The molar susceptibility χ_m is equal mass susceptibility χ_g multiplied by the formula weight of the substance.($\chi_m = \chi_g$ x molecular weight)

Here $(\mu_{eff}) = 2.828 \sqrt{\chi_m \times T}$

The magnetic moment was calculated using the above equation.

Table 2.1 : Unpaired spins and magnetic moments

No. of Unpared Electons	Total spin angular moment (S)	Magnetic moments μ_s (B.M)
1	1/2	1.73
2	1	2.83
3	1.5	3.87
4	2	4.90
5	2.5	5.92

The idea on magnetic measurements can be applied to understand the stereochemistry of metal complex. For instant $[Co(H_2O)_6]^{2+}$ and $[CoCl_4]^{2-}$. Effective magnetic momentum for octahedral complexes around room temperature are between 4.7 and 5.2 BM. For tetrahedral complexes the ground state acquires angular momentum 3.89, where 3.89 is the spin only-moment for three unpair electrons.

2.1.6. Metal Estimation

A known weight of the complex was taken into a conical flask and to it concentrated, H_2SO_4 (0.5) was added. It was fumed down to dryness and the process was repeated. Conc. HNO_3, (0.5ml) was then added and conc. $HClO_4$ (0.5 ml) were added too. The mixture was fumed to dryness. The process of the adding acids and fuming down to dryness was added to dissolve the sresidure and then the metal was estimated complexometrically[166]. Using EDTA (EDTA = Ethylenediamine tetraacetic acid) and DMG (DMG = Dimethyl glyoxime) excellent agreement of result were found.

2.1.7 Elemental analysis

Micro analysis for carbon hydrogen and nitrogen were obtained by using Kjeldahl Method.[167]

2.2 PURIFICATION OF THE SOLVENT[167]

2.2.1 Ethanol

About 1.25 g of clean and dry magnesium turnings and 0.125 g of iodine were placed in dry 500 mL round bottom flask containing 40 mL of reagent ethanol. The flask was then fitted with a condenser carrying a calcium chloride guard tube on the top. The mixture was warmed until, the iodine had disappeared: heating was continued until all the magnesium was converted into ethoxied, then 250 mL of absolute ethanol was added and then the mixture was refluxed for one hour. After cooling, the ethanol was distilled directly into a vessel in which it was stored, by resembling the condenser for downward distillation via a splash head adapter. Then the dry ethanol was collected into a receiving flask from which it was stored into an air tight bottle.

2.2.2 Acetone

Acetone was heated under reflux with successive quantities of potassium per manganese until the violet coloured persisted. It was dried with anhydrous potassium carbonate, filted from the desiccant and distilled. Precaution was taken to exclude moisture, i.e. a calcium chloride guard tube was used.

2.3 NAME OF THE CHEMICALS/REAGENTS USED AND SUPPLIERS

Cobalt (II) Perchlorate Hexahydrate	E. Merck, Germany
Zinc (II) Perchlorate Hexahydrate	E. Merck, Germany
Cadmiun (II) Perchlorate Hexahydrate	E. Merck, Germany
Cadmium(II) Carbonate	E. Merck, Germany
Cobalt(II) Carbonate	E. Merck, Germany
Zinc(II) Carbonate	E. Merck, Germany
Chloroform	E. Merck, Germany
Acetone	E. Merck, India
Methanol	BDH, England
Absolute ethanol	BDH, England
Dimethyl Sulfooxide	E. Merck, Germany
Formaldehyde	James Burrough Ltd. (England)
Acetaldehyde	BDH, England
Cinnamaldehyde	BDH, England
rotonaldehyde	E. Merck, Germany
Hydrazine Hydrate	E. Merck, Germany
Dimethylformamide(DMF)	BDH, England
Diethyl malonate	May & Baker Ltd.

All chemicals were used as supplied except purification the solvents which are purified in the laboratory.

Chapter - 3

EXPERIMENTAL

EXPERIMENTAL

3.2   PREPARATION OF COMPLEXES

3.1.1 Preparation of malonodihydrazide[137] $C_3H_8N_4O_2$

Diethyl malonate (8.080 g, 50 mmol) and hydrazine hydrate (5.006 g, 100 mmol) were mixed together in a beaker with constant stirring at ambient temperature. The reaction was carried out without solvent. The precipitate was formed when the solution was turned from yellowish to white. After the precipitation the product was filtered off on a buckner funnel and washed with ethanol three times and dried in a vacuum desiccator over anhydrous CaCl$_2$. A silky white amorphous product of 8.800g (60% yield) was obtained. The melting point of the compound was recorded at 125°C-130°C.

The substance was soluble in water and dimethyl formamide(DMF) and insoluble in ethanol, methanol, diethylether, petether carbontetrachloride and *n*-hexane.

Reaction of the preparation of the ligand:

Dimethyl malonate   Hydrazine hydratre   Malonodihydrazide
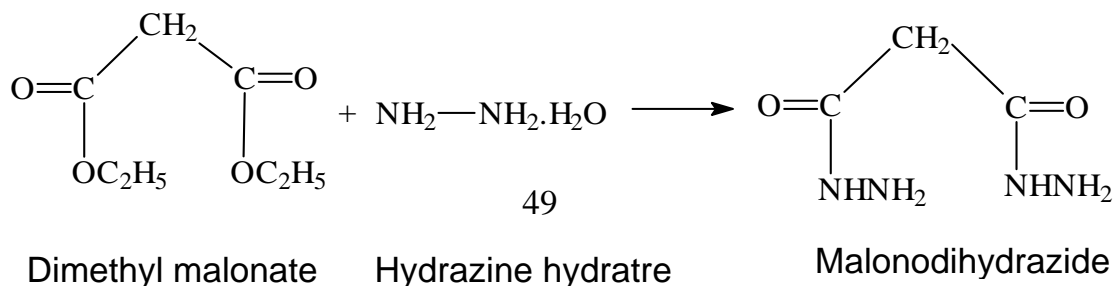
3.1.2 Preparation of Metal Perchlorate

Metal perchlorate was prepared from analytically pure metal carbonate by treatment with 70% perchloric acid. The treatment was done in the following way:

About 100 mL perchloric acid was taken in a 250 mL beaker and then metal carbonate (e.g. cadmium carbonate) was added slowly with continuous stirring. The addition of cadmium carbonate was continued until the bubbles were disappeared. Access amount of cadmium carbonate was added and kept it for 12 hours to ensure the completion of the reaction. Then water and alcohol mixture was added with continuous stirring. The resulting solids were filtered, washed with ethanol till free from excess acid and recrystallized several times from ethanol. Cadmium perchlorate is partially soluble in alcohol. Its solution was standardized by iodometric titration.

Cobalt perchlorate and zinc perchlorate were prepared with the same method.

3.1.3 PREPARATION OF MACROCYCLIC COMPLEXES OF Cd(II)

3.1.3.5 Preparation of [Cd($C_8N_8O_4H_{16}$)(ClO_4)$_2$] Macrocyclic Complex 1.

To the aqueous malonodihydrazide($C_3H_8N_4O_2$) (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 with ethanol for three times and dried in a vacuum desiccator over anhydrous $CaCl_2$. The melting point of the compound was 270°C (decompose) and yield was 1.290 g (76%). The compound was soluble in DMSO and insoluble in acetone, ethanol, water and chloroform.

3.1.3.2 Preparation of [Cd($C_{10}N_8O_4H_{20}$)(ClO_4)$_2$] Macrocyclic complex 2.

To the solution of malonodihydrazide ($C_3H_8N_4O_2$) (0.528 g, 4 mmol in 10 mL water), acetaldehyde (0.176 g, 4 mmol in 10 mL water) solution was added. To the above mixture, cadmium(II) perchlorate hexahydrate solution (0.839

g, 2 mmol in 10 mL water) was added and the whole mixture was refluxed with constant stirring for 5 hours and cooled down. A Pale yellow colour precipitate was formed. The precipitate was filtered off on a buckner funnel and was washed with water and ethanol for three times. The product was dried in a vacuum desiccator over anhydrous $CaCl_2$.

The melting point of the compound was 255°C (decompose) and yield was 0.950g (58.5%). The compound was soluble in DMSO and insoluble in acetone, alcohol, chloroform.

3.1.3.3 Preparation of $[Cd(C_{24}N_8O_4H_{28})(ClO_4)_2]$ Macrocyclic complex 3.

To the solution of malonodihydrazide $(C_3H_8N_4O_2)$ (0.528 g, 4 mmol in 10 mL water), cinnamaldehyde (0.528 g, 4 mmol in 10 mL water) 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 8 hours and cooled down. A Pale brown precipitate was formed. The precipitate was filtered off on a buckner funnel and was washed with water and ethanol for three times. The product was dried in a vacuum desiccator over anhydrous $CaCl_2$.

The melting point of the compound was 250°C (decompose) and yield was 0.97g (51%). The compound was soluble in DMSO and insoluble in chloroform, dimethyl formamide and acetone.

3.1.3.4 Preparation of $[Cd(C_{14}N_8O_4H_{24})(ClO_4)_2]$ Macrocyclic complex 4.

To the solution of malonodihydrazide $(C_3H_8N_4O_2)$, (0.528 g, 4 mmol in 10 mL water), crotonaldehyde (0.280 g, 4 mmol in 10 mL water) 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 12 hours and cooled down. Offwhite precipitate was formed. The product was filtered off on a buckner funnel and was washed with water and ethanol for three times and dried in a vacuum desiccator over anhydrous $CaCl_2$.

The melting point of the compound was 200°C (decompose) and yield was 1.11g, (70%). The compound is soluble in DMSO and insoluble in chloroform, dimethyl formamide, ethanol, carbontetrachloride and acetone.

3.1.4 PREPARATION OF MACROCYCLIC COMPLEXES OF Co(II)

3.1.4.2 Preparation of $[Co(C_8N_8O_4H_{16})(ClO_4)_2]$ Macrocyclic Complex 5.

To the solution of malonodihydrazide $(C_3H_8N_4O_2)$, (0.528 g, 4 mmol in 10 ml water), formaldehyde (0.320 g, 4 mmol 37%) solution was added. To the above mixture, cobalt(II) perchlorate hexahydrate (0.732g, 2 mmol in 10 mL water) solution was added and the hole mixture was refluxed with constant stirring for 4 hours and cooled down. A Light pink precipitate was filtered off on a buckner funnel. The product was washed with water and ethanol for three times. The product was dried in a vacuum desiccator over anhydrous

$CaCl_2$.

The melting point of the compound was 245°C (decompose) and yield was 0.96 g (61%). The compound was soluble in DMSO and insoluble in acetone, alcohol, chloroform and carbon tetrachloride.

3.1.4.2 Preparation of $[Co(C_{24}N_8O_4H_{28})(ClO_4)_2]$ Macrocyclic complex 6.

Malonodihydrazide ($C_3H_8N_4O_2$), (0.528 g, 4 mmol in 10 mL water) solution and cinnamaldehyde (0.528 g, 4 mmol in 10 mL water) solution were mixed together. To the above mixture, cobalt(II) perchlorate hexahydrate (0.732g, 2 mmol in 10 mL water) solution was added and the whole mixture was refluxed with constant stirring for 7 hours and cooled down to room temperature. A Light brown precipitate was formed. The product was filtered off on a buckner funnel and was washed with water and ethanol for three times and dried in a vacuum desiccator over anhydrous $CaCl_2$.

The melting point of the compound was 230°C (decompose) and yield was 0.98 g (55%). The compound was soluble in DMSO and insoluble in acetone, water, and chloroform.

Reaction of the preparation of the complexes:
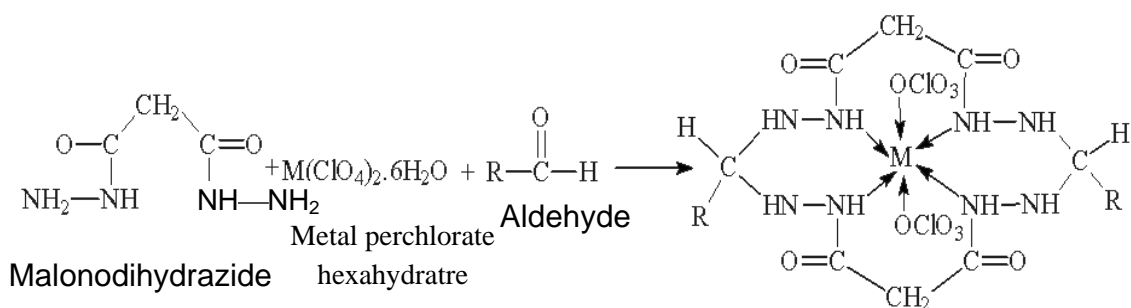
Where, R=H, CH_3, ⬡—CH=CH, CH_3CH=CH
M = Cd(II), Co(II)

3.1.5 ATTEMPTED REACTIONS

3.1.5.1 Preparation of [Co(C$_{10}$N$_8$O$_4$H$_{20}$)(ClO$_4$)$_2$] Macrocyclic complex.

To the solution of malonodihydrazide (0.528 g, 4 mmol in l0 ml water), acetaldehyde solution (0.176 g, 4 mmol in 10 mL water) was added. To the above mixture, cobalt(II) perchlorate hexahydrate (0.732g, 2 mmol in 10 mL water) solution was added and the whole mixture was refluxed with constant stirring for 12 hours. The colour of the mixture was not changed and no precipitate was formed. TLC suggested the starting materials.

3.1.5.5   Preparation of [Zn(C$_8$N$_8$O$_4$H$_{16}$)(ClO$_4$) $_2$] Macrocyclic Complex.

To the solution of malonodihydrazide (0.528 g, 4 mmol in l0 ml water), formaldehyde (0.320 g, 4 mmol 37% in 10 mL water) solution was added. To the above mixture, zinc(II) perchlorate hexahydrate (0.745 g, 2 mmol in 10 ml water) solution was added and the whole mixture was refluxed with constant stirring for 12 hours. The colour of the mixture was remaining same and no precipitate was formed. TLC suggested the starting materials.

3.1.5.6   Preparation of [Zn(C$_{10}$N$_8$O$_4$H$_{20}$)(ClO$_4$)$_2$] Macrocyclic Complex.

To the solution of malonodihydrazide (0.528 g, 4 mmol in 10 mL water), acetaldehyde solution (0.176 g, 4 mmol in 10 mL water) was added. To the above mixture, zinc(II) perchlorate hexahydrate solution (0.745 g, 2 mmol in 10 ml water) was added and the whole mixture was refluxed with constant stirring for 12 hours. The colour of the mixture was not changed and no precipitate was formed. TLC suggested the starting materials.

3.1.5.4 Preparation of $[Zn(C_{24}N_8O_4H_{28})(ClO_4)_2]$ Macrocyclic Complex

Malonodihydrazide, $C_3H_8N_4O_2$ (0.528 g, 4 mmol in 10 mL water) solution and cinnamaldehyde solution (0.528 g, 4 mmol in 10 mL water) were mixed together. To the above mixture, zinc(II) perchlorate hexahydrate (0.745 g, 2 mmol in 10 mL water) was added and the whole mixture was refluxed with constant stirring for 12 hours and cooled down to room temperature. The colour of the mixture was not changed and no precipitate was formed. TLC suggested the starting materials.

Chapter - 4

RESULTS

&

DISCUSSION

RESULT AND DISCUSSION

4.1 MACROCYCLIC COMPLEXES OF Cd(II)

Reactions of malonodihydrazide with Cd(II) perchlorate hexahydrate in the presence of formaldehyde, acetaldehyde, cinnamaldehyde and crotonaldehyde give some 16- member macrocyclic complexes described in sec. (3.1.3.1-3.1.3.4).

Complexes **(1-4)** are characterized on the basis of elemental analysis, conductance measurements, UV-visible spectra and infrared studies and other physical properties, like melting point, solubility, colour etc.

Molar conductance data of the complexes **(1-4)** are shown in Table 4.2. The conductance values of the complexes suggested that they are non- electric in nature[127,136].

The infrared spectra of the complexes **(1-4)** are shown in Fig (4.1-4.4). The infrared spectral data (Table 4.3) of the complexes showed a strong and broad band at (3222-3261) cm⁻¹ which is assigned to the ν(NH) stretching[168-170] . Due to coordination the ν(N-H) stretching of amide group goes to the higher

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frequency at (3222, 3261) cm^{-1} region as compared to the starting material malonodihydrazide[178]. In the complexes the terminal-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 ν(N-H) bands at (3248, 3213,3050) cm^1. The bands at (3248, 3050) cm^{-1} for the asymmetric and symmetric ν(N-H) stretching of the terminal-NH$_2$ moiety and 3213 cm^1 for the ν(N-H) stretching of the amidic(N-H) group].

The complexes showed a broad band at (2912-3047) cm^{-1} which may be assigned to the ν(C-H) stretching of aliphatic moiety[171] . The complexes showed a strong band (1652-1685) cm^{-1} which represent the ν(C=O) of

$$NH-NH-\overset{\overset{\textstyle O}{\textstyle \|}}{C}-CH_2$$ moiety[171] . Three or four bands at (621-1143) cm^{-1} region also indicated the ν_1 , ν_2 , ν_3, ν_4 bands of (ClO$_4^{-1}$) moiety. These stretching frequencies suggested the coordination of perchlorate to the metal through the O atom[126, 127,134]. A medium band at (416-443) cm^{-1} region is tentatively attributed to the ν(M-N) mode[168-170] indicating the coordination of the metal through the nitrogen atom.

The elemental analysis (C, H and N) (Table 4.1) and metal estimated data (Table 4.2) of the complexes are consistent with the proposed formula (Fig. 4.9).

The UV-visible spectra of the complexes (1-4) are shown in Fig. (4.5-4.8). The complexes showed bands (Table 4.4) 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}A$_{2g}$(F) $\rightarrow$ ^{3}T$_{1g}$(F), ^{3}A$_{2g}$(F) $\rightarrow$ ^{3}T$_{1g}$(P), which suggested the octahedral geometry of the Cd(II) complexes[172,173].

On the basis of elemental analysis, conductance measurements, infrared and UV spectra studies and other physical properties, the suggested structure of the complexes are octahedral in nature as shown in Fig. 4.9.
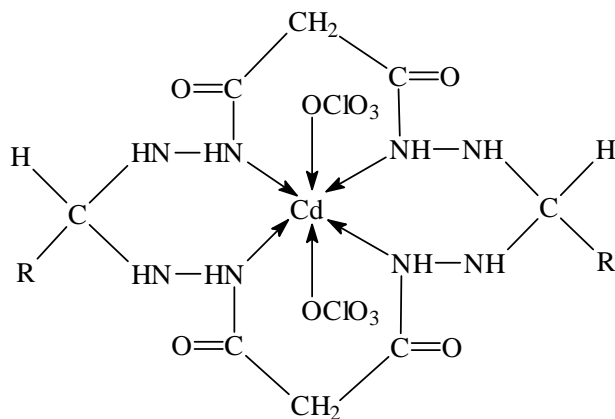
Where, R = H, (**1**); CH$_3$(**2**); ⬡—CH=CH, (**3**); CH$_3$CH=CH(**4**)

Fig.: 4.9

Table-4.1: Data for **e**lemental analysis of compounds **(1-4)**.

No.	Compounds	% C		% H		% N	
		Calculated	Found	Calculated	Found	Calculated	Found
1	$[Cd(C_8N_8O_4H_{16})(ClO_4)_2]$	16.02	16.09	2.67	2.69	16.70	16.71
2	$[Cd(C_{10}N_8O_4H_{20})(ClO_4)_2]$	19.12	19.14	3.19	3.20	17.86	17.79
3	$[Cd(C_{24}N_8O_4H_{28})(ClO_4)_2]$	35.83	35.90	3.49	3.50	13.95	13.97
4	$[Cd(C_{14}N_8O_4H_{24})(ClO_4)_2]$	24.74	24.76	3.53	3.44	16.49	16.30

Table-4.2: Magnetic moment data of compounds (**1-4**). T=301K

No.	Compounds	Sample length, l in cm	Weight of the sample, m in gm	Susceptibility of the empty tube, R_o	Susceptibility of the sample with test tube, R	Mass Susceptibility, χ_g $\times 10^{-6}$ C.G.S. unit	Molecular weight, M	Molar Susceptibility, $\chi_m \times 10^{-3}$ C.G.S. unit	μ_{eff} B.M.
1	$[Cd(C_8N_8O_4H_{16})(ClO_4)_2]$	2.1							
2	$[Cd(C_{10}N_8O_4H_{20})(ClO_4)_2]$								
3	$[Cd(C_{24}N_8O_4H_{28})(ClO_4)_2]$								
4	$[Cd(C_{14}N_8O_4H_{24})(ClO_4)_2]$								

Table-4.2: Analytical data and other physical properties of compounds **(1-4)**.

No.	Compounds	Color	Melting points °C	M% Calculated	M% Found	Molar conductance $Ohm^{-1}cm^2mol^{-1}$	% yield
1	$[Cd(C_8N_8O_4H_{16})(ClO_4)_2]$	White	210(d)	17.941	18.760	2.0	76%
2	$[Cd(C_{10}N_8O_4H_{20})(ClO_4)_2]$	Pale yellow	270(d)	17.064	17.928	3.18	58%
3	$[Cd(C_{24}N_8O_4H_{28})(ClO_4)_2]$	Pale brown	200(d)	14.052	13.998	1.87	51%
4	$[Cd(C_{14}N_8O_4H_{24})(ClO_4)_2]$	Offwhite	286(d)	16.021	16.555	1.33	70%

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

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Table-4.3: Important infrared spectral bands of compounds **(1-4)**.

No.	Compounds	$\nu(\text{N-H})\text{cm}^{-1}$	$\nu(\text{C-H})\text{cm}^{-1}$	$\nu(\text{C=O})\text{cm}^{-1}$	$\nu(\text{ClO}_4)\text{cm}^{-1}$	$\nu(\text{M-N})\text{cm}^{-1}$
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

Table-4.4: UV-visible absorption maxima of compounds **(1-4)**.

No	compounds	$\lambda_{max \; (nm)}$
1	$[Cd(C_8N_8O_4H_{16})(ClO_4)_2]$	350, 400
2	$[Cd(C_{10}N_8O_4H_{20})(ClO_4)_2]$	350, 400
3	$[Cd(C_{24}N_8O_4H_{28})(ClO_4)_2]$	370, 550
4	$[Cd(C_{14}N_8O_4H_{24})(ClO_4)_2]$	350, 400
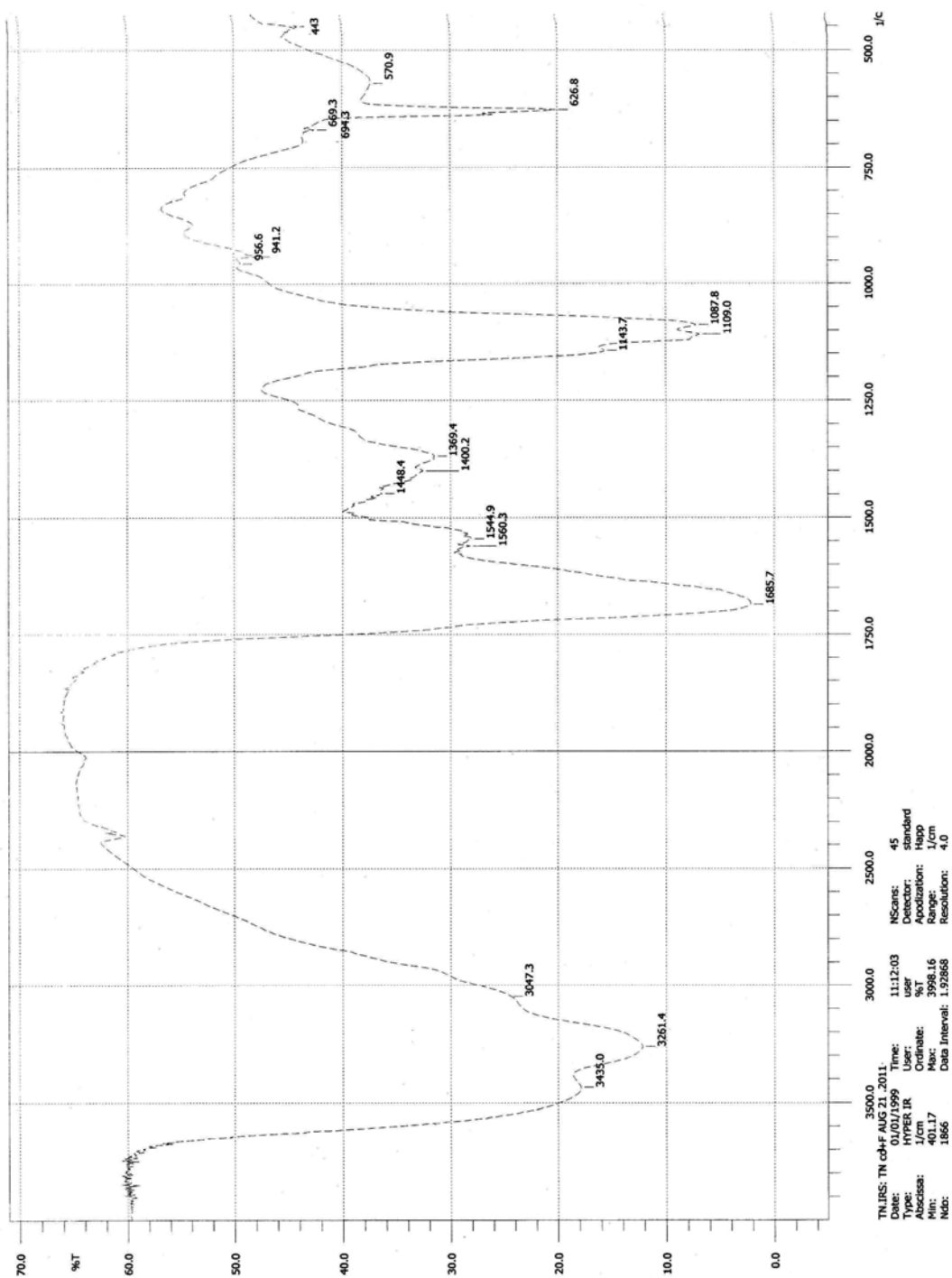
Fig-4.1: Infrared spectrum of $[Cd(C_8N_8O_4H_{16})(ClO_4)_2]$complex **1.**
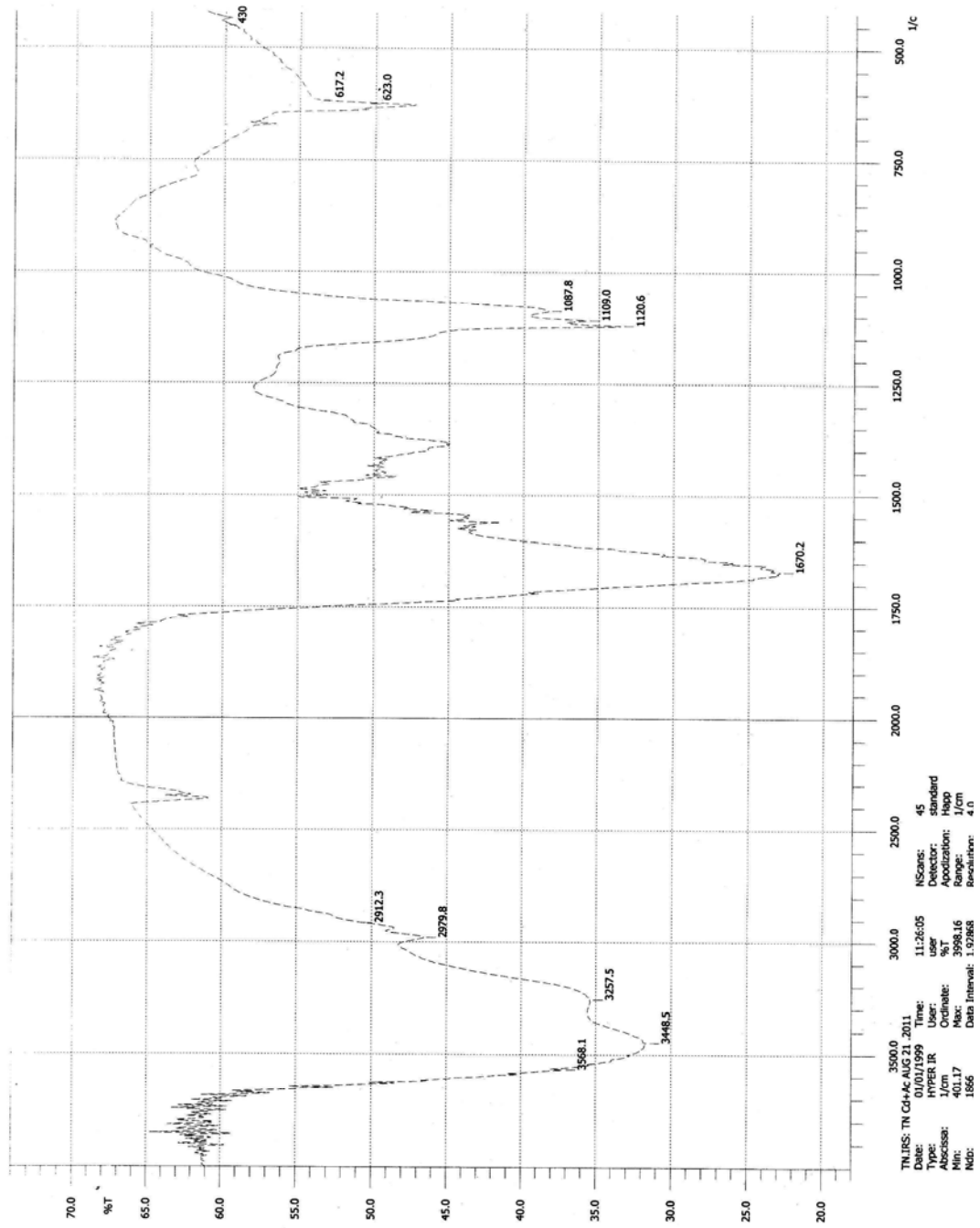
Fig-4.2: Infrared spectrum of [Cd(C$_{10}$N$_8$O$_4$H$_{20}$)(ClO$_4$)$_2$]complex **2.**
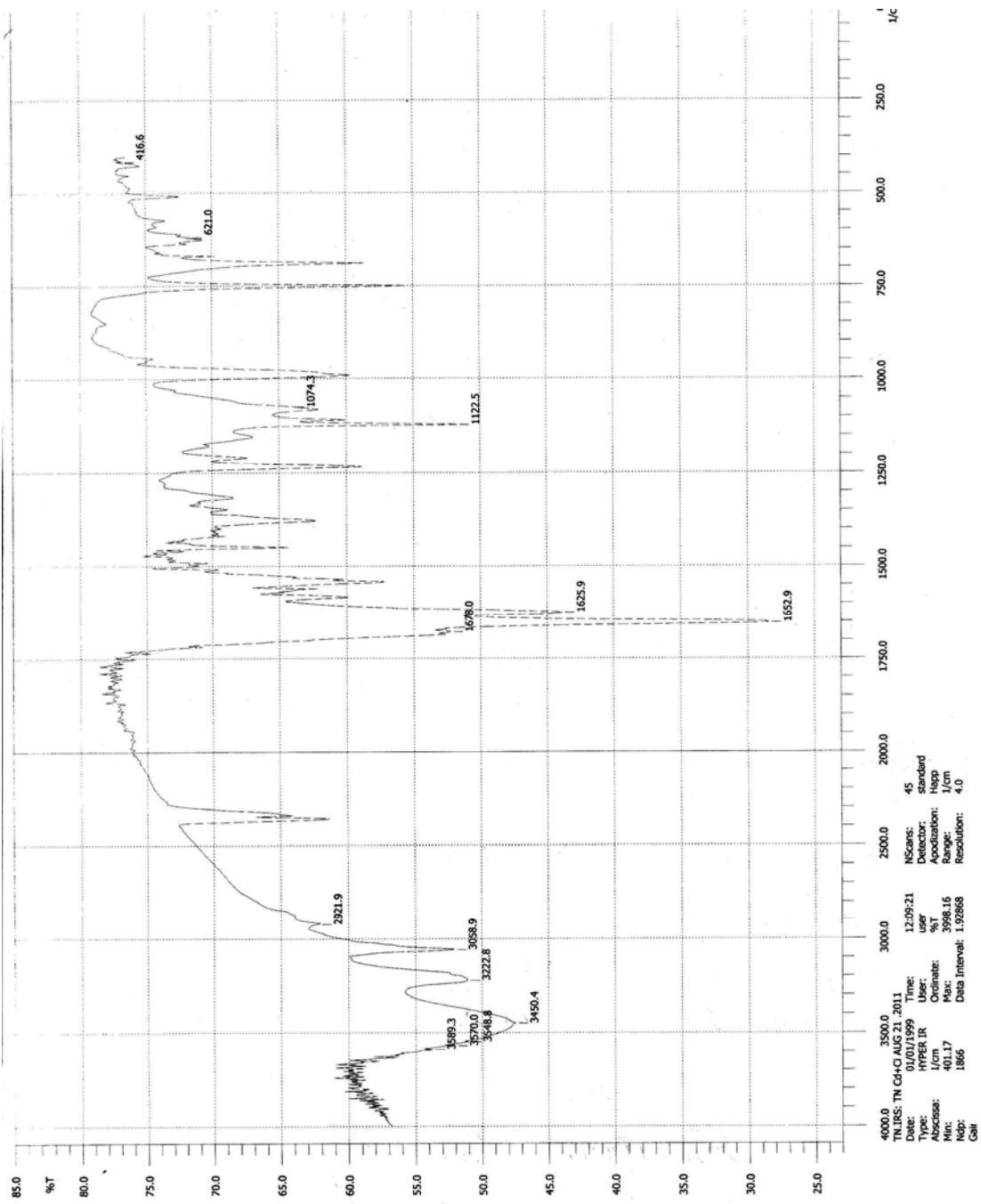
Fig-4.3: Infrared spectrum of $[Cd(C_{24}N_8O_4H_{28})(ClO_4)_2]$complex **3.**

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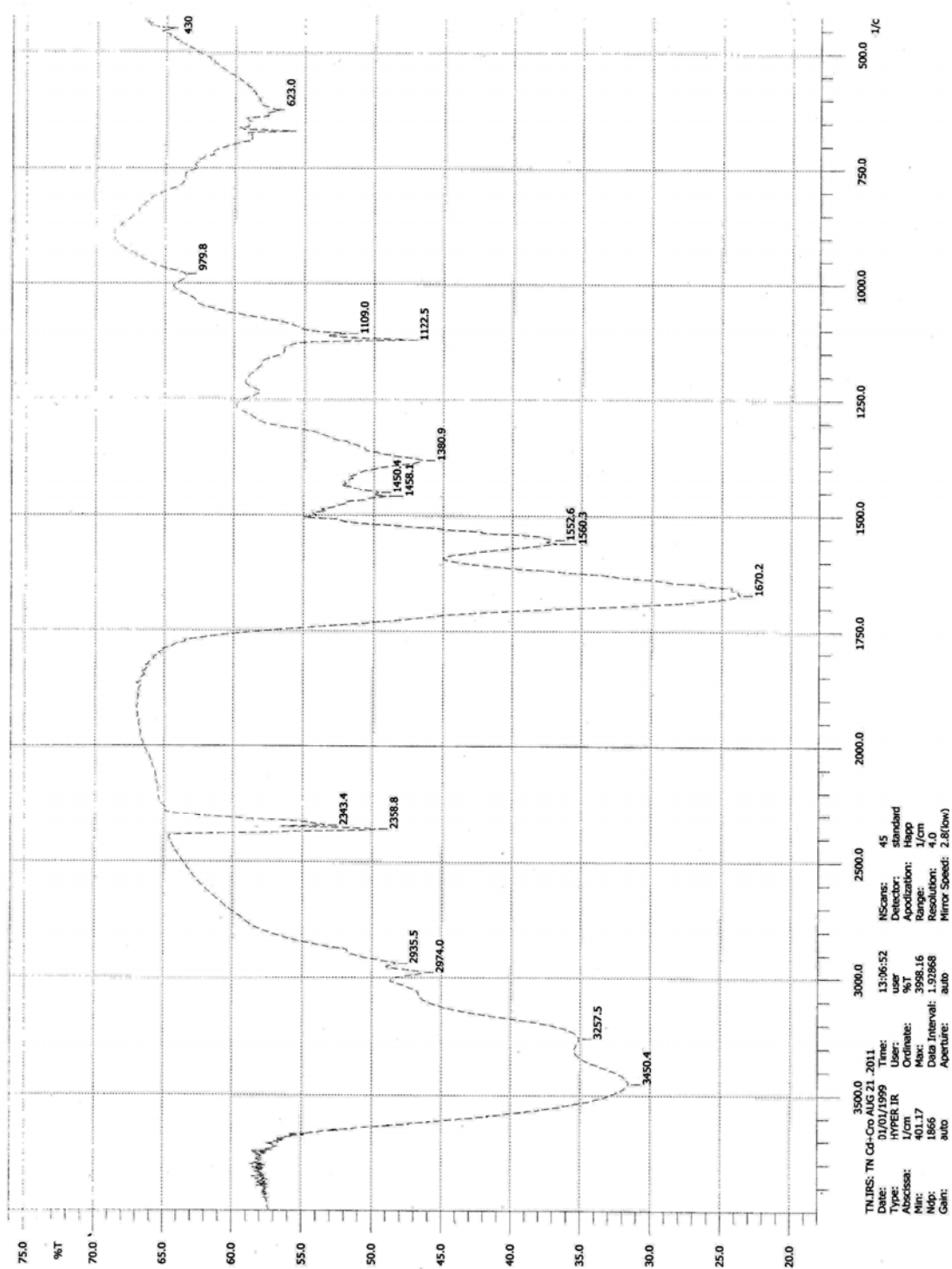
Fig-4.4: Infrared spectrum of [Cd(C$_{14}$N$_8$O$_4$H$_{24}$)(ClO$_4$)$_2$] complex **4.**
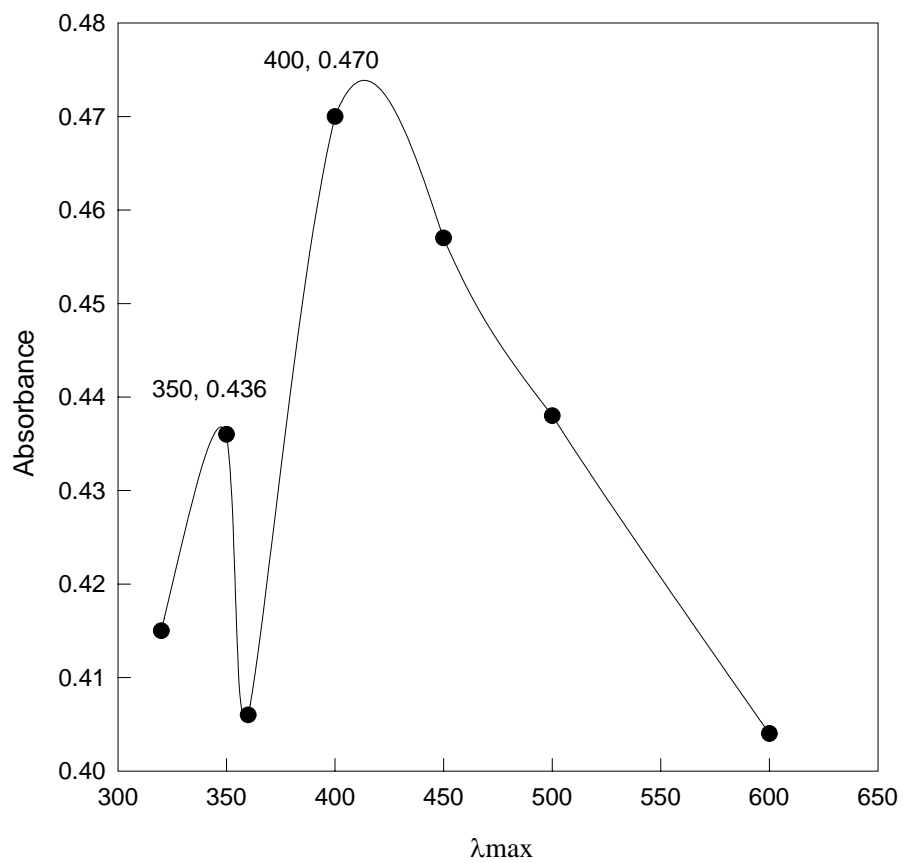
Fig. 4.5: UV-visible spectrum of [Cd(C$_8$N$_8$O$_4$H$_{16}$)(ClO$_4$)$_2$]   complex **1.**
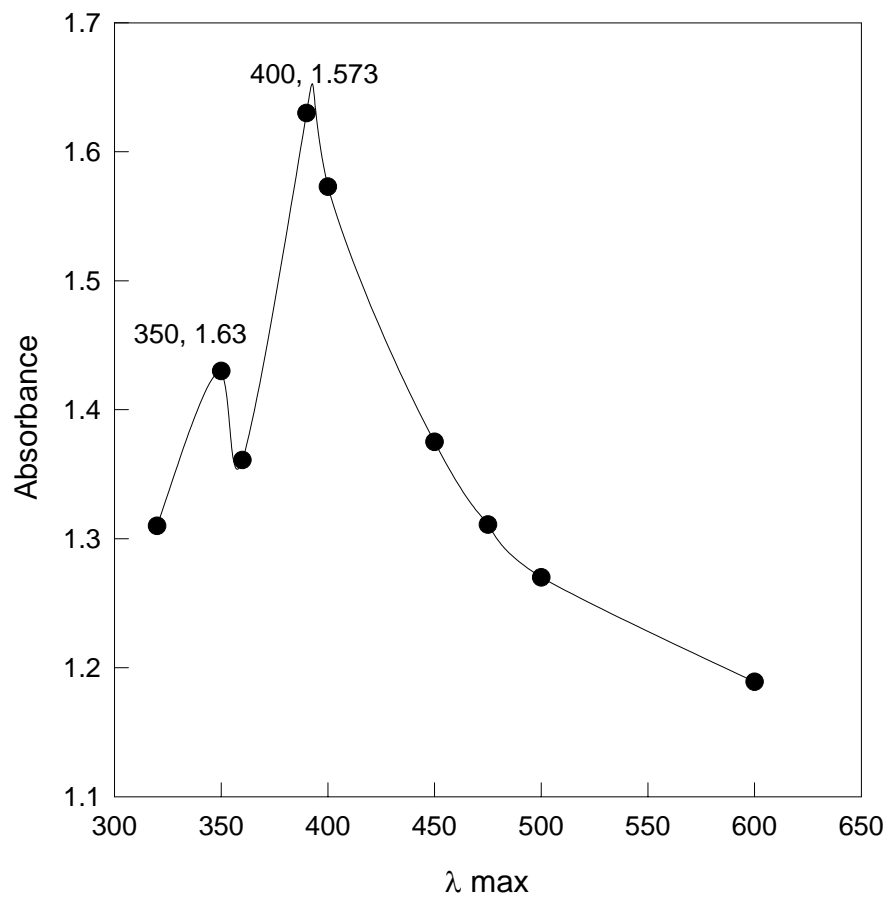
Fig-4.6: UV-visible spectrum of [Cd(C$_{10}$N$_8$O$_4$H$_{20}$)(ClO$_4$)$_2$] complex **2.**
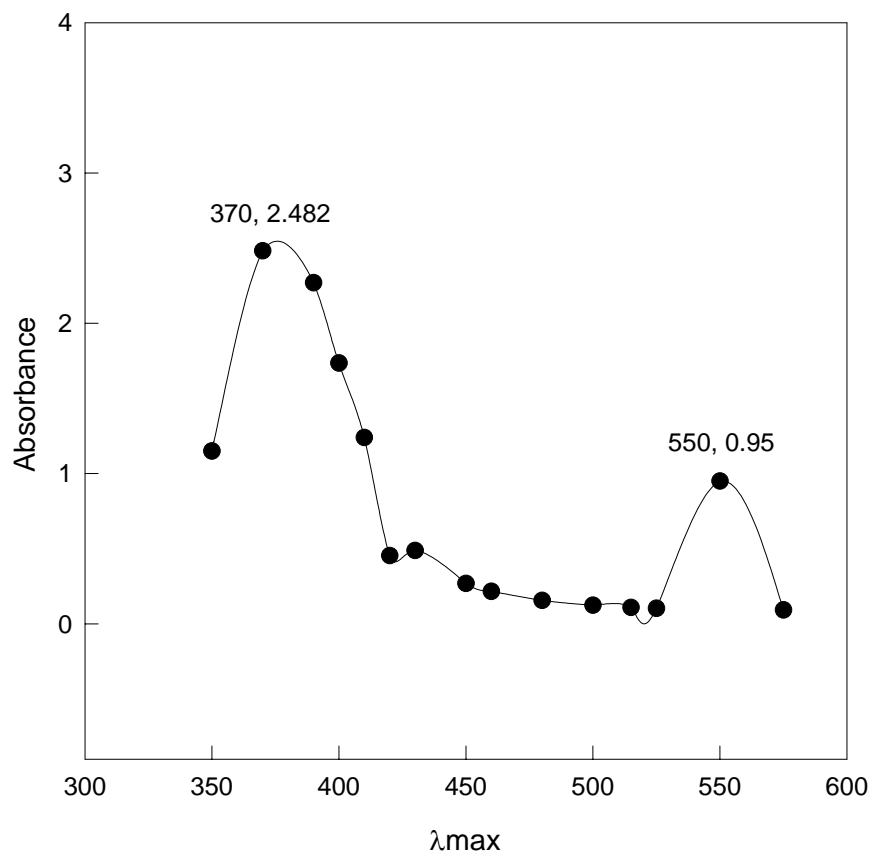
Fig-4.7: UV-visible spectrum of [Cd(C$_{24}$N$_8$O$_4$H$_{28}$)(ClO$_4$)$_2$] complex **3.**
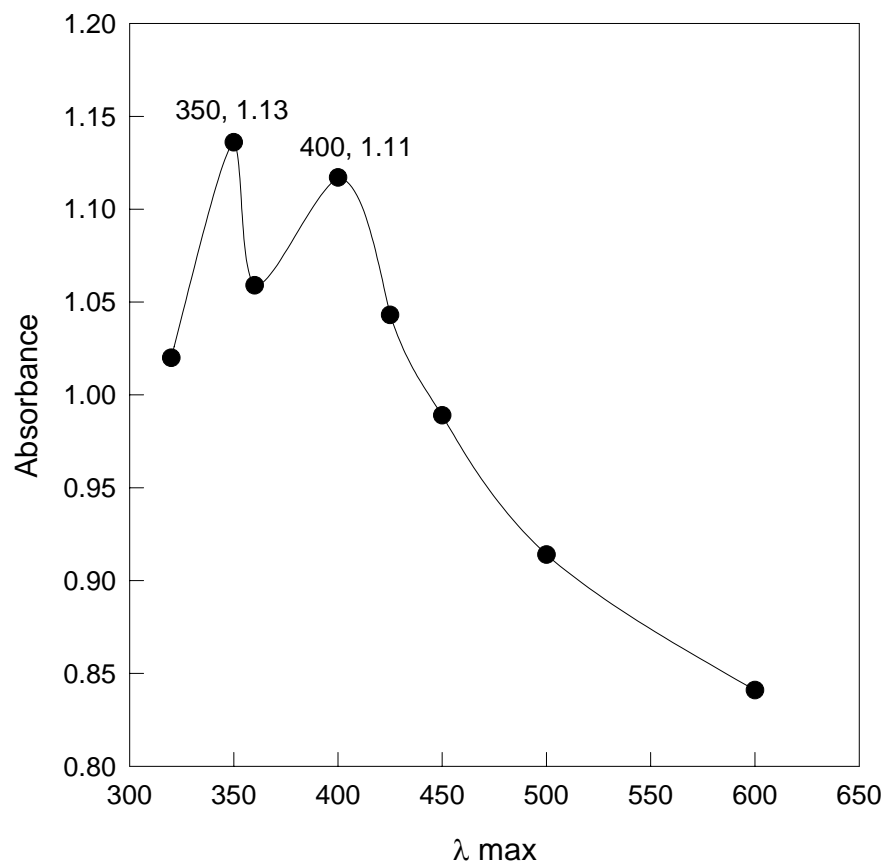
Fig-4.8: UV-visible spectrum of $[Cd(C_{14}N_8O_4H_{24})(ClO_4)_2]$ complex **4.**

4.2 MACROCYCLIC COMPLEXES OF Co(II).

Reactions of malonodihydrazide with Co(II) perchlorate hexahydrate in the presence of formaldehyde and cinnamaldehyde give some 16- member macrocyclic complexes described in sec. (3.1.4.1-3.1.4.2).

Complexes **(5-6)** are characterized on the basis of elemental analysis, magnetic moment & conductance measurements, UV visible spectra & infrared spectroscopy and other physical properties, like melting point, solubility, colour etc.

Molar conductance data of the complexes **(5-6)** are shown in Table 4.7. The conductance values of the complexes suggested that they are non-electrolytic in nature.[127,136]

The infrared spectra of the complexes **(5-6)** are shown in Fig (4.9-4.10). The infrared spectra data (Table 4.8) of the complexes showed a strong and broad band at (3211-3257) cm^{-1} which is assigned for the $v(NH)$ stretching[168-170].

Due to coordination the $v(N-H)$ stretching of amide group goes to the higher frequency at (3211, 3257) cm^{-1} region as compared to the starting material malonodihydrazide[178]. In the complexes the terminal $-NH_2$ group of malonodihydrazide condensed with aldehyde moiety and form a new secondary-NH group which may appear at the same region (or overlap) as to the amide-NH group, as a result the $v(N-H)$ band appear as a strong and broad band. [The starting material malonodihydrazide have three $v(N-H)$ bands at (3248, 3213,3050) cm^{1}. The bands at (3248, 3050) cm^{-1} for the

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asymmetric and symmetric ν(N-H) stretching of the terminal-NH_2 moiety and 3213 amidic (N-H) group].

The complexes showed a broad band at (2902-3008) cm⁻¹ which may be assigned to the ν(C-H) stretching mode of aliphatic moiety[171]. The complexes showed a strong band (1651-1676) cm⁻¹ which represents the ν

(C=O) of $NH-NH-\overset{\overset{\displaystyle O}{\|}}{C}-CH_2$ moiety[171]. Three or four bands at (613-1153) cm⁻¹ region also indicated the ν_1 , ν_2 , ν_3, ν_4 bands of (ClO_4^-) moiety. These stretching frequencies suggested the coordination of perchlorate to the metal through the O atom[126,127,134]. A medium band at (420-424) cm⁻¹ region is tentatively attributed to the ν(M-N) mode[168-170] indicating the coordination of the metal through the nitrogen atom.

The magnetic moment measurement data (Table 4.6) of the Co(II) complexes **(5-6)** showed values of (3.81-3.86) B.M. These values correspond to three unpaired electron of Co(II) d^7 system, suggested the octahedral environment of the complexes which are consistent with the literature value.

The elemental analysis (C, H and N) (Table 4.5) and metal estimation data (Table 4.6) of the complexes are consistent with the proposed formula.

The UV-visible spectra of the complexes **(5-6)** are shown in Fig. (4.11-4.13). The complexes showed bands (Table 4.9) at 400,500 nm **(5)**, at 390,550 nm, **(6)** represent the d-d transition of $^4T_{1g} \rightarrow {}^4T_{2g}$ for d^7, which suggested the octahedral geometry of the Co(II) complexes[176]. The band observed below 400nm assigned to the $\pi \rightarrow \pi^*$ transition[171,177] of the macrocyclic ligand.

On the basis of elemental analysis, magnetic moment and conductance measurements, infrared spectra & UV visible spectra and other physical properties, the suggested structure of the complex is shown in Fig. 4.13.
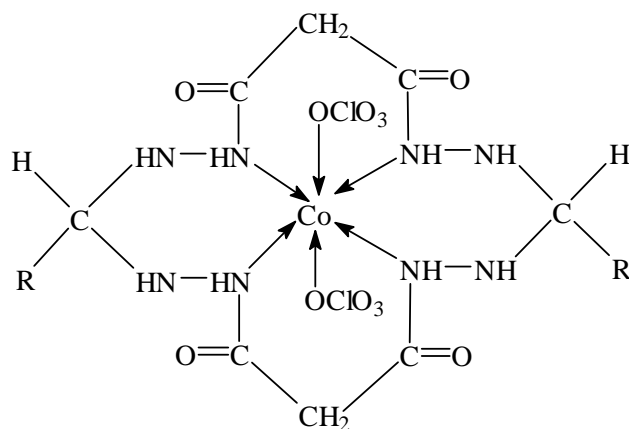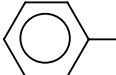
Where, R = H, (**5**); ⬡—CH=CH, (**6**);

Fig.: 4.13.

Table-4.5: Data for the elemental analysis of compounds (**5-6**).

No.	Compounds	% C		% H		% N	
		Calculated	Found	Calculated	Found	Calculated	Found
5	$[Co(C_8N_8O_4H_{16})(ClO_4)_2]$	17.58	17.67	2.93	2.94	20.51	20.50
6	$[Co(C_{24}N_8O_4H_{28})(ClO_4)_2]$	38.40	38.50	3.73	3.75	14.93	14.97

Table-4.6: Magnetic moment data of compounds (**5-6**). T=301K

No.	Compounds	Sample length, l in cm	Weight of the sample, m in gm	Susceptibility of the empty tube, R_o	Susceptibility of the sample with test tube, R	Mass Susceptibility, $\chi_g \times 10^{-6}$ C.G.S. unit	Molecular weight, M	Molar Susceptibility, $\chi_m \times 10^{-3}$ C.G.S. unit	μ_{eff} B.M.
5	$[Co(C_8N_8O_4H_{16})(ClO_4)_2]$	3.8	0.2650	-52	-15	11.067	546	6.043	3.81
6	$[Co(C_{24}N_8O_4H_{28})(ClO_4)_2]$	3.7	0.0187	-40	-20	8.255	750	6.191	3.86

Table-4.7: Analytical data and other physical properties of compounds (**5-6**).

No.	Compounds	Color	Melting points °C	M% Calculated	M% Found	Molar conductance Ohm^{-1}cm^{2}mol^{-1}	% yield
5	[Co(C$_8$N$_8$O$_4$H$_{16}$)(ClO$_4$)$_2$]	Light pink	290(d)	10.86	10.09	2.65	61%
6	[Co(C$_{24}$N$_8$O$_4$H$_{28}$)(ClO$_4$)$_2$]	Light brown	180(d)	7.86	6.95	1.24	55%

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

Table-4.8: Important infrared spectral bands data of compounds (**5-6**).

No	Compounds	ν(N-H)cm^{-1}	ν(C-H)cm^{-1}	ν(C=O)cm^{-1}	ν(ClO$_4$)cm^{-1}	ν (M-N)cm^{-1}
5	[Co(C$_8$N$_8$O$_4$H$_{16}$)(ClO$_4$)$_2$]	3257	3008	1676	1120,970,613	420
6	[Co(C$_{24}$N$_8$O$_4$H$_{28}$)(ClO$_4$)$_2$]	3211	2902	1651	1153,1076,623	424

Table-4.9: UV-visible absorption maxima of compounds (**1-4).**

No	Compounds	$\lambda_{max\ (nm)}$
5	$[Co(C_8N_8O_4H_{16})(ClO_4)_2]$	400, 500
6	$[Co(C_{24}N_8O_4H_{28})(ClO_4)_2]$	390, 550

78
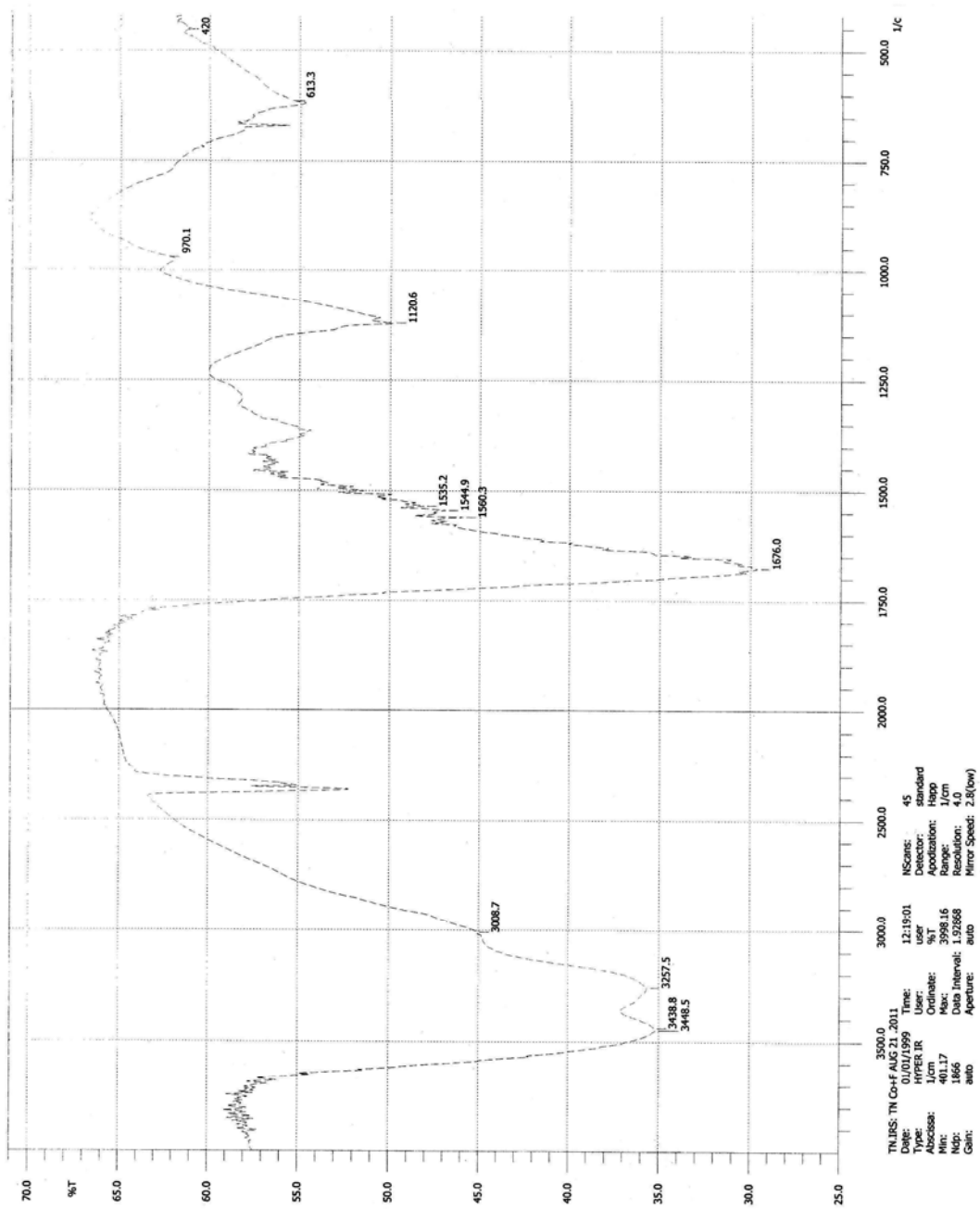
Fig-4.9. Infrared spectrum of $[Co(C_8N_8O_4H_{16})(ClO_4)_2]$ complex **5.**
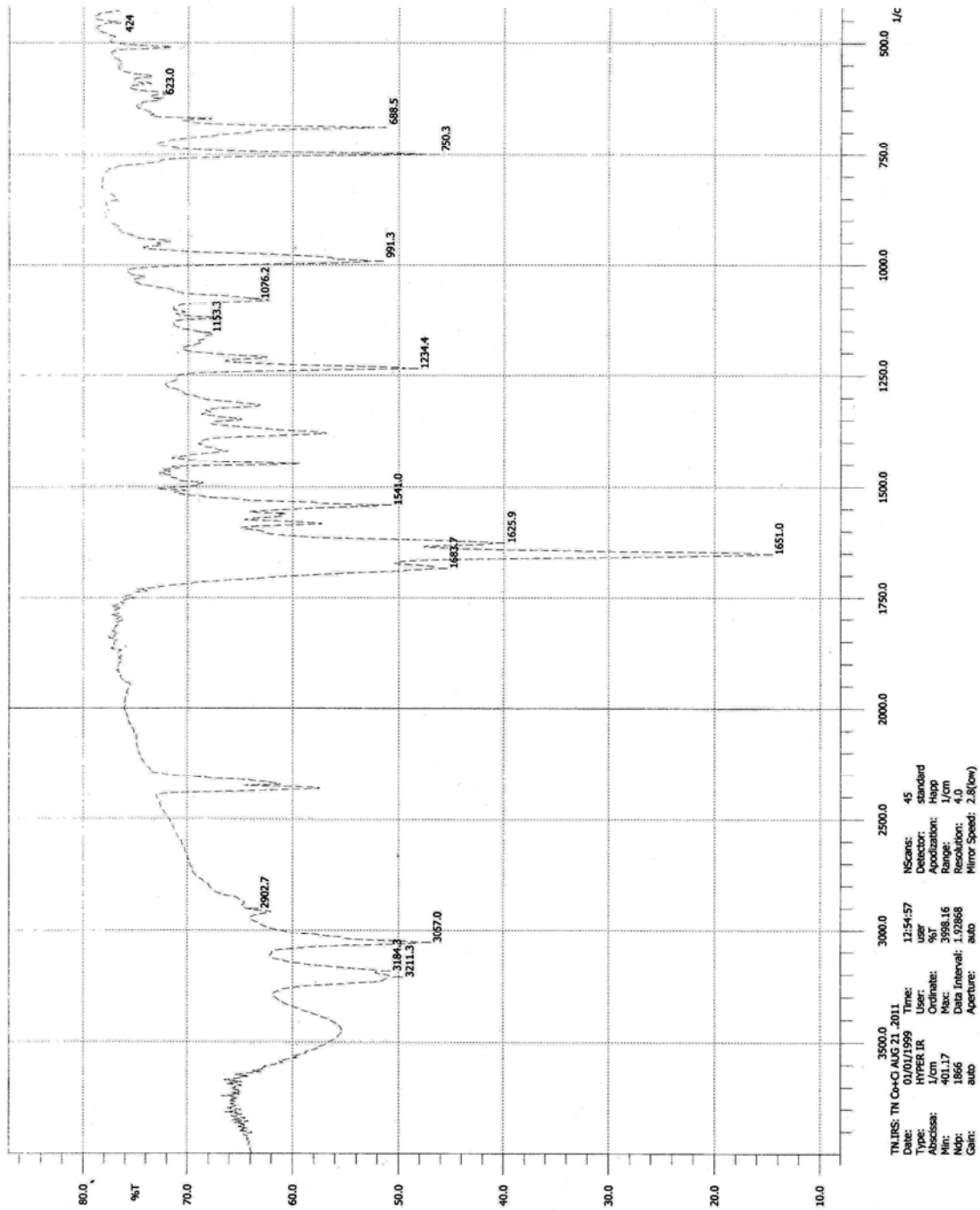
Fig-4.10: Infrared spectrum of $[Co(C_{24}N_8O_4H_{28})(ClO_4)_2]$complex **6.**
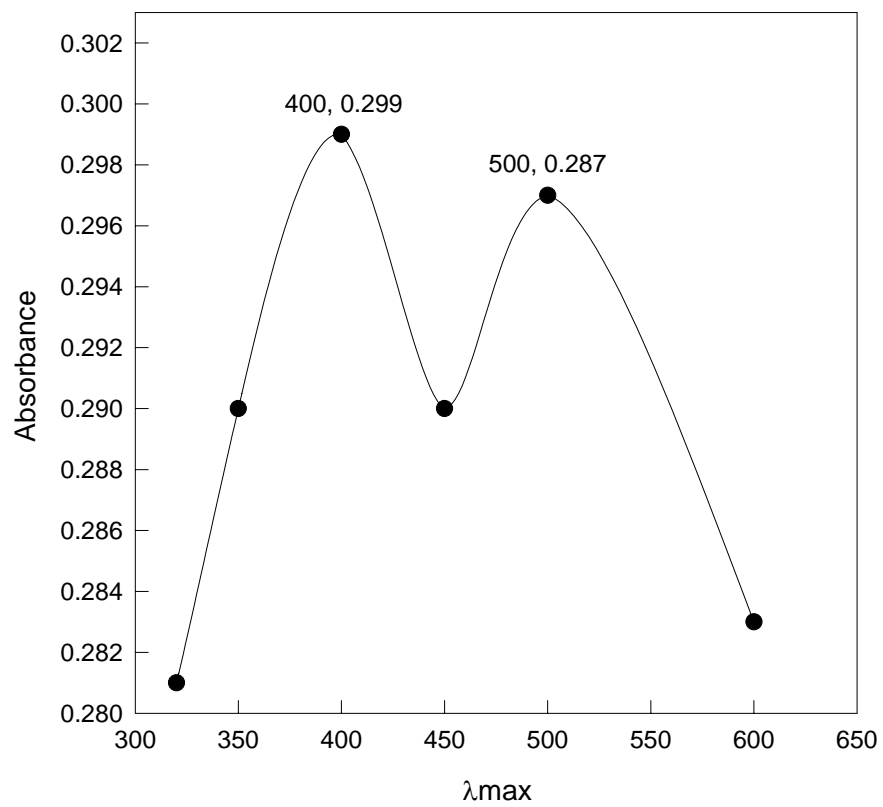
Fig. 4.11: UV-visible spectrum of $[Co(C_8N_8O_4H_{16})(ClO_4)_2]$ complex **5.**
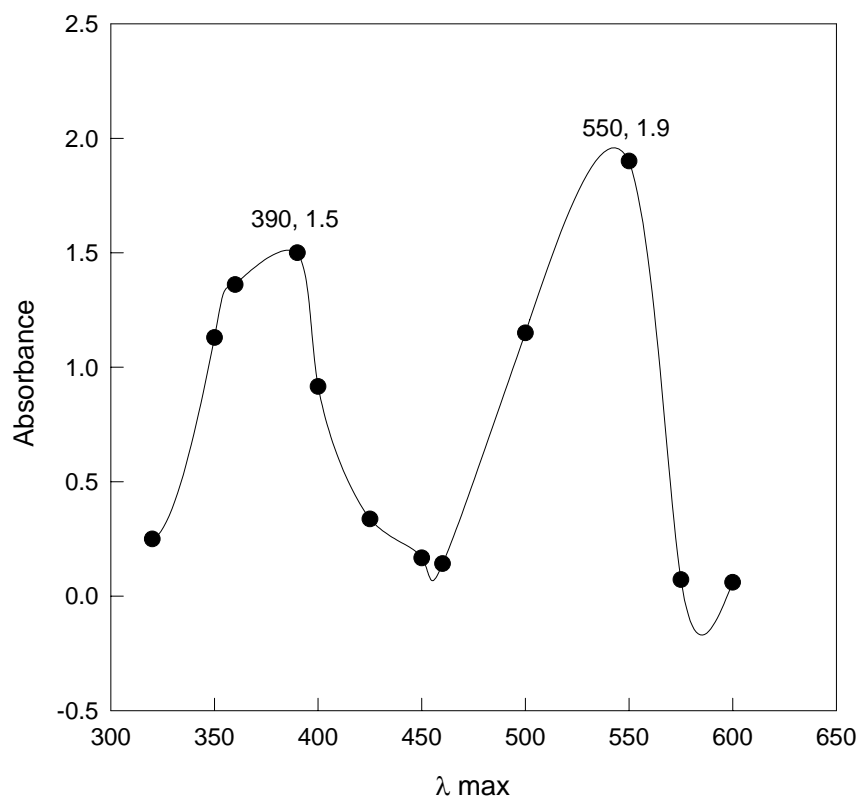
Fig-4.12: UV-visible spectrum of [Co(C$_{24}$N$_8$O$_4$H$_{28}$)(ClO$_4$)$_2$] complex **6.**

4.3 Macrocyclic complexes of Zn(II)

Reactions of malonodihydrazide with Zn(II) perchlorate hexahydrate in the presence of formaldehyde, acetaldehyde, cinnamaldehyde and crotonaldehyde were tried. But we were unable to prepare the macrocyclic complexes of Zn in our lab. Probabaly this is because of the size of metal ion. We know that the size of metal ion is very important in the formation of macrocyclic complexes. Formation of macrocyclic complexes decreases with the decrease of the size of metal ion. When the size of the metal is large, then the ligand can easily coordinate to the metal ion. The ionic radii of Zn(30), is smaller than that of Co(27) due to the lanthanide contraction. So, the tendency of complex formation of Zn is smaller than that of Co.

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Chapter - 5

ANTIBACTERIAL ACTIVITY

ANIBACTERRIAL ACTIVITIES

5.1 INTRODUCTION AND PRINCIPLE

An anti-microbial is a substance that kills or inhibits the growth of microorganisms[1] such as bacteria, fungi, or protozoans. Antimicrobial drugs either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic). Disinfectants are antimicrobial substances used on non-living objects or outside the body.

The history of antimicrobials begins with the observations of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth of another. They did not know at that time that the reason one bacterium failed to grow was that the other bacterium was producing an antibiotic. Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism. Of course, in today's common usage, the term antibiotic is used to refer to almost any drug that attempts to rid your body of a bacterial infection. Antimicrobials include not just antibiotics, but synthetically formed compounds as well.

Antimicrobial resistance is the ability of a microorganism to resist the effects of an antimicrobial agent. May be an intrinsic characteristic or acquired by selection for mutation or by acquisition of a resistance gene from other microorganisms.

Antimicrobial sensitivity test is an in vitro test of the effectiveness of selected antibacterial agents against bacteria recovered from a patient. Paper disks impregnated with various agents are placed on an inoculated agar plate (disc diffusion) or the agent is added to broth cultures.

Inhibition of growth is interpreted as an indication of bacterial sensitivity to the antibacterial.

The susceptibility of microorganism to antimicrobial agent can be determined in vitro by a number of methods. The disc diffusion technique[2,3] is widely acceptable for preliminary investigation of materials, which are suspected to posses antimicrobial properties. Diffusion procedure is normally used for inessential qualitative test, which allocates organism of the susceptible intermediate (moderately susceptible) or resistant categories.

The Dried filter paper discs containing the test materials are usually applied to the test plate containing the culture of microorganisms. These are kept at low temperature (4°C) for 24 hours.

Initially the dried discs absorb water from the surrounding test medium and the drug is dissolved. The drug migrates through the adjacent test medium by concentration gradient of the drug according to the physical law that governs diffusion of molecules through an agar gel[4]. As a result there is a gradual change of drug concentration in the agar surrounding each disc. Then the plates are incubated in an incubator at 37°C for 6 hours. Activities of test sample are expressed by measuring the zone of inhibition observed around the area of the disc.

As the antibiotic diffusion progresses microbial multiplication also proceeds. After an initial lag phase a logarithmic growth phase in initiated at that moment bacterial multiplication proceeds more rapidly than the drug can diffuse and the bacterial cell which are not inhibited by the antimicrobial agents will continue to multiply until a lawn of grown can

be visualized. No growth will appear in the area where drug is present in inhibitory concentration.

Generally more susceptibility the test organism the larger is the circular zone of inhibition. Antimicrobial activities of the test sample are expressed by the measuring the zone of inhibition observed around the area of the disc. The diameter of the inhibition is usually measured to understand the extent of inhibition in different concentration.

The size of the inhibitory zones depends on the following principle factors.

   i.   Intrinsic antimicrobial sensitivity of the test sample.
  ii.   Growth rate of the test microorganism
 iii.   Diffusion rate of the drug which is related to its water solubility.
 iv.   Number of concentration of the freshly seeded test organism.
  v.   Amount of the test sample on disc.
 vi.   Thickness of the test medium in the Petri dishes.
vii.   Thickness of the filter paper disc.

5.2 APPARATUS AND REAGENTS

      i.   Micropipette.
     ii.   Autoclave.
    iii.   Incubator.
    iv.   Refrigerator.
     v.   Filter paper disc.
    vi.   Petri dishes.
   vii.   Inoculation loop.
  viii.   Sterile cotton.
    ix.   Sterile forceps.
     x.   Spirit lamp.
    xi.   Laminar air flow unit.
   xii.   Nutrient agar.

5.3   METHOD

The test organisms are pathogenic for human beings. For this reason, all steps of the work were done with high precaution and aseptic condition which are mentioned below. All steps of work were carried out at microbiology laboratory at the Department of Microbiology in the University of Dhaka.

5.4   TEST OF ORGANISMS USED FOR THE STUDY

Five pathogenic bacteria were selected for the test, four of which were gram negative, and one was gram positive.

List of the Test Pathogenic Bacteria

> **<u>Gram Negative</u>**
> *Preudomonas aeruginosa*
> *Kicbsilla*
> *Shigella flexneri*
> *Escherichia coli*
>
> **<u>Gram Positive</u>**
> *Bacillus ccreus*

5.5 CULTURE MEDIA

Nutrient agar medium was used as culture media. The instant nutrient agar (DIFGCO) medium was weight and then reconstituted with distilled water in a conical flask according to pacification (2.3% w/v). The formulation of nutrient agar media (DIFCO) is as follows:

Nutrient agar (mast diagnostics)

Formulation	Grams/Litre
Peptone A	6.0
Yeast extract	2.0
Beef extract	1.0
Sodium chloride	5.0
Agar	14.0
Distilled water sq. to 1000 mL	

Total 28 grams of powder was weighed and dispersed in one litre of distilled water allowed to shake for 10 minutes, scoirled to mixed and then sterilized by autoclaving for 15 minutes at 121°C. Then medium was cooled to (40-45)°C and mixed well, then poured in to plates.

5.6   PREPARATION OF FRESH CULTURE

The liquid culture is called broth culture. The culture media per litre as follows:

Formulation	Grams/Litre
Bacto tryptone	10.0 g
Bacto yeast extract	5.0 g
NaCl	10.0 g

Adjusted pH to 7.5 with sodium hydroxide.

Tryptone, NaCl and yeast extract of calculated amount were taken in a conical flask and distilled water was added (volume should be less than 1 litre) the contents were heated in water bath to make a clear solution. The pH of the solution was then adjusted to 7.5 using NaOH or HCl as necessary. Distilled water was added sufficiently to make the final volume (1litre). Again the total volume was heated on a water bath to obtain a clear solution. The conical flask was plugged with cotton and then autoclaved at 1 atm pressure for 15 minutes at 120°C.

50 mL of broth medium was transferred in a conical flask. The test microorganisms of pure culture were streaked on the nutrient broth media with the help of sterile loop in an aseptic condition and incubated at 37°C for 24 hours. The broth culture thus obtained was considered as fresh culture. Fresh culture of this type was always used throughout the sensitivity testing.

5.7 PREPARATION OF THE CULTURE PLATE

A small bottle containing 10 mL sterile nutrient broth was taken and the test organism (*Bacillus megaterium*) from the pure culture transferred to this bottle with the help of an inoculation loop in an aseptic condition. After inoculation at 37°C for 24 hours to provide sufficient time and temperature for the growth of the test organism.

To 100 mL of the nutrient agar 1 mL of the prepared culture was added and was mixed thoroughly with shaking. A 25 mL portion of this culture was poured in to a petridish and in order to faeifitate homogeneous distribution of the test organism, the petridish was rotated several times first in clockwise direction and then in anti clockwise direction. The media were pouring in to petridish on a level horizontal surface so as to give a uniform depth of approximately 4 nm. The petridish was undistributed for about 15 minutes during which it was solidified. After complete solidified of the media 4-5 holes were made inside it with the help of a brother.

Just before using with lids agar were place in an incubator (25°C) for about 10-15 minutes until the execs of surface moisture was lost by evaporation. There should be no droplets of on observing their antibacterial activities the species *Bacillus megaterium*, was taken as test organism.

5.8   PREPARATION OF DISCS

A. Sample discs

    i.   Solution of the compounds was prepared in respective solvents so that 20µL contained 200µg of the compounds.

    ii.   Filter paper disc were taken in petridish and sterilized by oven at 110°C for 1 hours.

    iii.   20µL of the solutions were placed on the discs with the help of a micropipette thus discs containing 200 µg compounds were prepared.

    iv.   These discs were then air-dried.

B. Standard Disc

Ready made kanamycin K-30 µg/disc of antibiotic kanamycin was used as standard disc.

5.9 PLACEMENT OF THE DISC AND INCUBATION

The solidified agar plates were seeded with the 200 µL of fresh culture with the help of a micropipette and spread the microorganisms with the help of a sterile spreader in an aseptic condition.

The prepared disc of samples was placed gently on the freshly seeded solidified agar plates with a sterile forceps. Standard disc were also placed on the test plate to compare the effect of the test sample and to nullify the effect of solvent respectively.

The plates were then kept in a refrigerator at 4°C for 24 hours in order that the materials had sufficient time to diffuse to a considered area of plates. After this the plate plates were incubated at 37°C for 6 hours.

5.10 CALCULATION OF THE ZONE OF INHIBITION

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition.

After incubation the diameter of the zone inhibitions were observed and, measured in mm by a transparent scale, result, obtained from these are listed in the table (5.1-5.6).
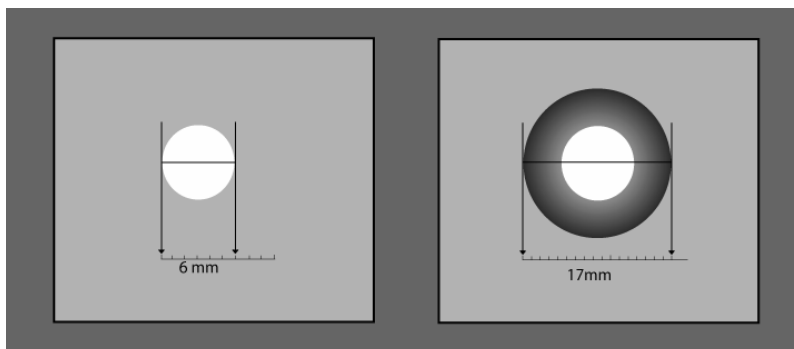
Fig 5: Determination of the zone of inhibition

5.11: RESULT OF THE ANTIBACTERIAL ACTIVITY OF THE COMPLEXES **(1-6)** AGAINST THE FIVE PATHOGENIC BACTERIA VIZ. *KICBSILL, SHIGELLA FLEXNERI, ESCHERICHIA COLI, PSEUDOMONAS AERUGINOSA, BACILLUS CCREUS.*

Table 5. Complexes abbreviation for antibacterial activity.

Complexes No.	Compound	Symbol
7	$[Cd(C_{24}N_8O_4H_{28})(ClO_4)_2]$	Cd+Cin+L
8	$[Cd(C_{10}N_8O_4H_{20})(ClO_4)_2]$	Cd+A+L
9	$[Co(C_{24}N_8O_4H_{28})(ClO_4)_2]$	Co+Cin+L
Ligand	$C_3N_4H_8O_2$	L

Table 5.1: Antibacterial activity of the complexes **7(3)** and **8(2)** against *Preudomonas aeruginosa.*

Complex No	Symbol	Zone of inhibition mycelia growth (in mm)			
		200µg/disc	100µg/disc	50µg/disc	30µg/disc
7	Cd+Cin+L	-	-	-	-
8	Cd+A+L	-	-	-	-
	Control disc	Nil		-	-
	Standard disc				25

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Table 5.2: Antibacterial activity of the complex **7(3)** against *Bacillus ccreus.*

Complex No	Symbol	Zone of inhibition mycelia growth (in mm)			
		200µg/disc	100µg/disc	50µg/disc	30µg/disc
7	Cd+Cin+L	15	8	-	-
	Control disc	Nil		-	-
	Standard disc				22

Table 5.3: Antibacterial activity of the complex **7(3**) against *Shigella flexneri.*

Complex No	Symbol	Zone of inhibition mycelia growth (in mm)			
		200µg/disc	100µg/disc	50µg/disc	30µg/disc
7	Cd+Cin+L	-	-	-	-
	Control disc	Nil		-	-
	Standard disc				18

Table 5.4: Antibacterial activity of the complex **9(6)** against *Klcbsilla.*

Complex No	Symbol	Zone of inhibition mycelia growth (in mm)			
		200µg/disc	100µg/disc	50µg/disc	30µg/disc
9	Co+Cin+L	-	-	-	-
	Control disc				
	Standard disc				20

Table 5.5: Antibacterial activity of the complex **9(6)** against *Escherichia coli.*

Complex No	Symbol	Zone of inhibition mycelia growth (in mm)			
		200µg/disc	100µg/disc	50µg/disc	30µg/disc
9	Co+Cin+L	-	-	-	-
	Control disc	Nil		-	-
	Standard disc				18

Table 5.6: Antibacterial activity of the complexes **7(3)** & **8(2)** against *Klcbsilla*.

Complex No	Symbol	Zone of inhibition mycelia growth (in mm)			
		200µg/disc	100µg/disc	50µg/disc	30µg/disc
7	Cd+Cin+L	-	-	-	-
8	Cd+A+L	-	-	-	-
	Control disc	Nil		-	-
	Standard disc				21

Fig. 5.1: Photographic representation of zone of inhabitation of the complexes **7(3), 8(2)** and the standard compound kanamycin against *Preudomonas aeruginosa.*

Table 5.11: Abbreviation of the photographic labeling sample.

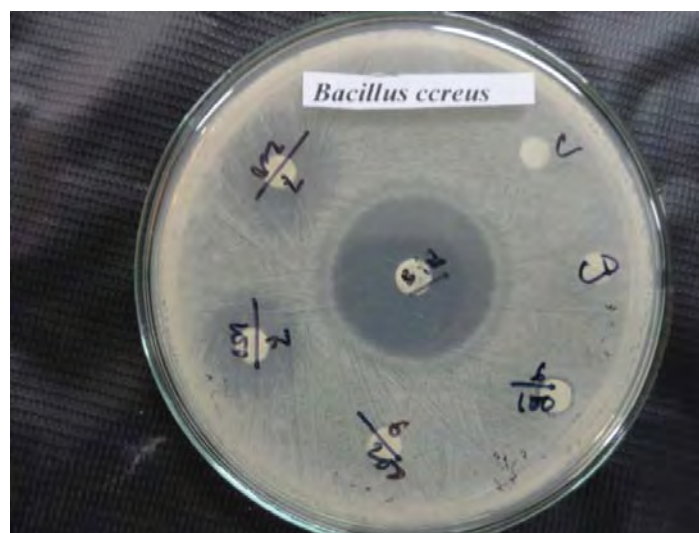| Complex No	symbol	Labels	Concentration µg/disc
2	Cd+A+L	8	100
			200
3	Cd+Cin+L	7	100
			200
6	Co+Cin+L	9	100
			200

Fig. 5.2: Photographic representation of zone of inhabitation of the complex **7(3)** and the standard compound kanamycin against *Bacillus ccreus*.
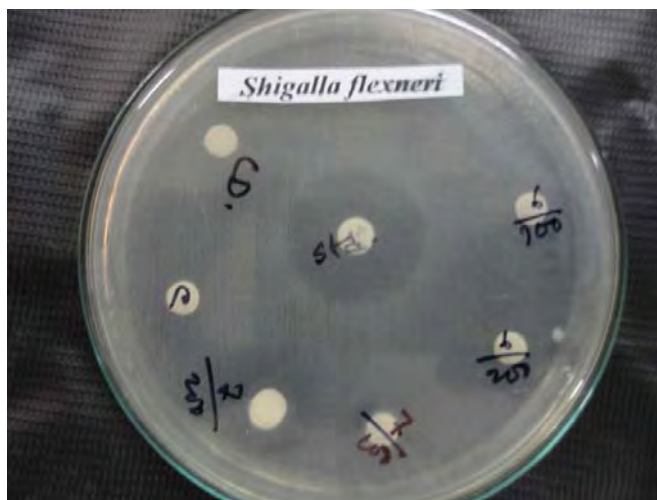
Fig. 5.3: Photographic representation of zone of inhabitation of the complex **7(3)** and the standard compound kanamycin against *Shigella flexneri*.

Fig. 5.4: Photographic representation of zone of inhabitation of the complexe **9(6)** and the standard compound kanamycin against *Klcbsilla*.

Fig. 5.5: Photographic representation of zone of inhabitation of the complexe **9(6)** and the standard compound kanamycin against *Escherichia coli*.

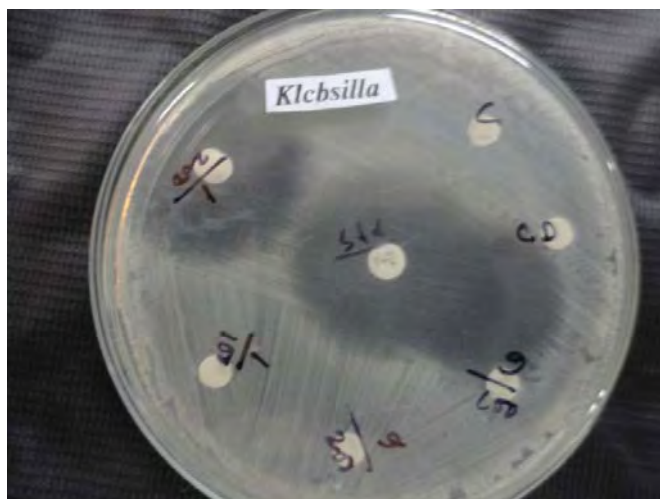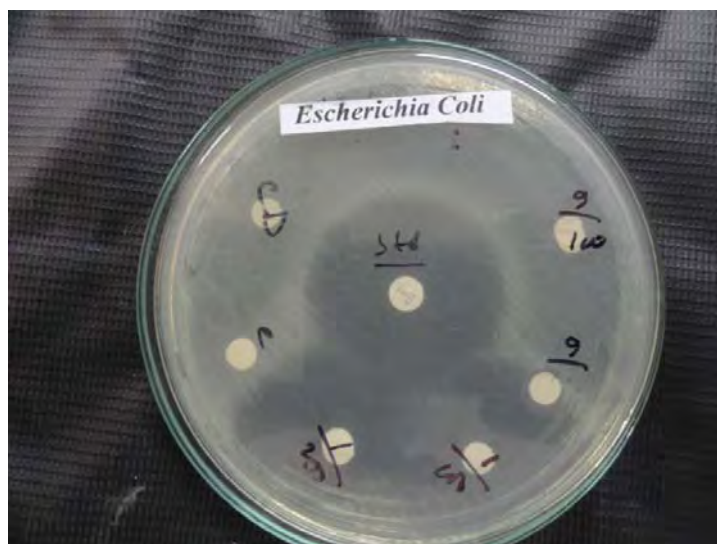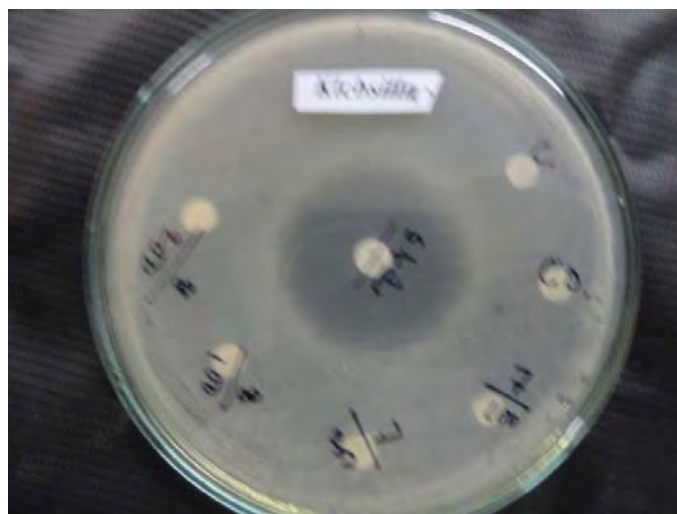**Fig. 5.6:** Photographic representation of zone of inhabitation of the complexes **7(3), 8(2)** and the standard compound kanamycin against *Klcbsilla*.

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5.12 Discussion

It has been observed that some drug increases the activity when administered as metal complexes or their metal chalets[5]. The antibacterial activity of the metal complexes **7(3)**, **8(2)**, and **9(6)** complexes are recorded against five pathogenic bacteria viz.

Gram negative

1. *Preudomonas areruginosa*
2. *Klcbsilla*
3. *Shigella flexneri*
4. *Escherichia coli*

Gram positive

5. *Bacillus ccreus*

And the results are given in (Table 5.1-5.6) the complex **7(3)** showed the most activities above five pathogenic bacteria as shown Table (Fig 5.1-5.6). 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 5.1-5.6).

The good activity against *Bacillus ccreus* and less active *Klcbsilla* 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.

5.13   DETERMINATIONOF   MINIMUM   INHIBITORY CONCENTRATIONS (MIC) OF COMPLEXES

5.13.1 Introduction

Minimum inhibitory concentration (MIC) may be defined as the lowest concentration of antimicrobial drug to inhibit the growth of organism. The data derived from the test can be corrected with the knowledge of expected or measured antibiotic level in vivo to predict the efficacy of the sample.

There are two methods for determining the MIC are as follows.
  i.   Serial dilution technique or turbidimetric assay[6,7].
  ii.   Paper disc technique or agar diffusion assay[6]**.**

Here "serial dilution technique"[6,7] was followed using nutrient broth medium. The MIC values of complexes were determined against the following five-test organisms.

Gram negative
  1. *Preudomonas areruginosa*
  2. *Klcbsilla*
  3. *Shigella flexneri*
  4. *Escherichia coli*

<u>**Gram positive**</u>

    5.   *Bacillus ccreus*

5.13.2 Principle of serial tube dilution technique

The tubes of broth medium containing graded doses of sample are inoculated with the test organisms. After suitable incubation growth will occur in these inhibitory tubes were the concentration of sample is bellow the inhibitory level and the culture will become turbid. Therefore the large number of microorganism present growth will not occur above the inhibitory level and the tube will remain clear.

5.13.3 Preparation of the sample solution

2.048 mg of the test compound was taken in a vial 2 mL of methanol was added to the vial to dissolve the compound. Thus solutions with a concentration of 1.024 mg/mL were obtained. The solution was added the medium on the serial dilution.

5.13.4 Preparations of inoculums

Fresh culture of the test organisms was grown at 37°C for overnight on nutrient agar medium. Bacterial suspensions were then prepared in sterile saline TS and the turbidity was adjusted with saline TS to obtain the turbidity visually comparable to that of Farland 0.5 standard. The bacterial suspension was further diluted to 1:200 in Muller hinton broth. The resulting suspension contained 106 $_{CFU/mL}$.

5.13.5 Procedure

i. 12 test tubes were taken nine of which were marked 1, 2, 3, 4, 5, 6, 7, 8, 9 and the rest three were assigned as CM (nutrient broth medium), Cs (nutrient broth medium + Compound), Ci [nutrient broth medium inoculums (organism)].

ii. 1 mL of nutrient broth medium was poured to each of the 12 test tubes.

iii. These test tubes were cotton plugged and sterilized in an autoclave for 15 minutes at 121°C temperature and 1 atm pressure.

iv. After cooling 1 mL of the sample solution was added to the 1st tube mixed well and then 1 mL of this content was transferred to the second test tube.

v. The content of the second test tube was mixed well and again 1 mL of this mixture was transferred to the 3rd tube this process of serial dilution was continued up to the 9th test tube and 1 mL mixed content was discarded from 9th test tube.

vi. 10μL of properly diluted inoculums was added to each of six test tubes and mixed well.

vii. To the control test tube, C_s 1 mL of the sample solution was added mixed well and 1mL of this mixed content was discarded this is to check the clearity of the medium in presence of diluted.

viii. 10 μL of the inoculums was added to the control test tube C_i to observe the growth of the organism in the medium used.

ix. The control test tubes C_M containing medium only was used to confirm the sterility of the medium.

x. All the test tube was incubated at 37° for 20 hours MIC is the lowest drug concentration at which there is no growth.

5.14 RESULT OF MINIMUM INHIBITORY CONCENTRATION OF THE COMPLEX [Cd(C$_{24}$N$_8$O$_4$H$_{28}$)(ClO$_4$)$_2$] AGAINST THE FIVE PATHOGENIC BACTERIA VIZ.

Gram negative

1. *Preudomonas areruginosa*
2. *Klcbsilla*
3. *Shigella flexneri*
4. *Escherichia coli*

Gram positive

5. *Bacillus ccreus*

Table 5.7: Minimum inhibitory concentration of [Cd(C$_{24}$N$_8$O$_4$H$_{28}$)(ClO$_4$)$_2$] complex, **7(3)** against *Bacillus ccreus.*

Test tube No	Nutrient broth medium added (mL)	Diluted solution of complex (µg/mL)	Inoculum added (µL)	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$_s$	1	512	0	-ve
C$_i$	1	0	10	+ve
C$_M$	1	0	0	-ve

The MIC of the complex **7** is 128 µg/mL

5.15 Discussion

The minimum inhibitory concentration (MIC) of the complexes $[Cd(C_{24}N_8O_4H_{28})(ClO_4)_2]$ as **7(3)** was determined against five pathogenic bacteria viz.

1. ***Preudomonas areruginosa***

2. ***Klcbsilla***

3. ***Shigella flexneri***

4. ***Escherichia coli***

5. ***Bacillus ccreus***

by serial dilution technique.

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