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Antimicrobial Potential of Transition Metal Hexacyanoferrates†

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Six transition metal hexacyanoferrates namely manganese, cobalt, nickel, copper, zinc and cadmium ferrocyanides have been synthesized and characterized on the basis of IR, elemental analysis, magnetic susceptibility measurement and thermal analysis. Antibacterial screening of synthesized metal ferrocynides has been carried out against *Escherichia coli* 101, *Escherichia coli* 119, *Pseudomonas fluorescence*, *Micrococcus luteus, Bacillus subtilis* and *Bacillus cereus*. *M. luteus causes asthma* while *B. subtilis* and *B. cereus* cause food poisoning. Rest of the other strains are non pathogenic. Copper ferrocyanide has shown potential activity against *E. coli* 119, *E. coli* 101 and *B. cereus*. Cobalt ferrocyanide has been found to be most effective against *B. subtilis.* Cadmium ferrocyanide has shown significant activity against *M. luteus* and *P. fluorescence*.

Key Words: Transition metal complexes, Hexacyanoferrates, Antibacterial activities.

INTRODUCTION

Many transition metal ions in the living systems work as enzymes or carriers in macrocyclic ligand field environment. Therefore meaningful research in this direction might generate simple models for biologically occurring metallo enzymes[1] and thus will help in developing our understanding of biological systems. These ligands are also of theoretical interest as they are capable of furnishing an environment of controlled geometry and ligand field strength[2,3]. Literature survey disclosed that a number of polydentate macrocyclic ligands and their metal complexes have been reported[4].

Transition metals have an important place within medicinal biochemistry. Research has shown significant progress in utilization of transition metal complexes as drugs to treat several human diseases like carcinomas, lymphomas, infection control, antiinflammatory, diabetes and neurological disorders[5,6]. Transition metals exhibit different oxidation states and can interact with a number of negatively charged molecules. This activity of transition metals has started the development of metalbased drugs with promising pharmacological application and may offer unique therapeutic opportunities. To provide an update on recent advances in the medicinal use of transition metals, a Medline search was undertaken to identify the recent relevant literature[7].

There are few reports on antifungal and antibacterial activity of transition metal ferrocyanides in literature[8]. These complexes may possess antimicrobial activity against pathogenic bacteria being used as a test organism in the present study.

Experiments of primitive earth conditions, cyanide could have combined with a large number of metal ions present in primeval sea. Consequently, several insoluble metal ferrocyanides of general formula $M_2\,[Fe\,(CN)_6]\cdot H_2O$, where M = Mn, Co, Ni, Cu, Zn and Cd *etc*. could have been formed. It is well established that metal ferrocyanides acts as adsorbent[9], ion-exchangers and photosensitizers.

These synthesized transition metal complexes have been screened for their activity against *Escherichia coli* 101. *Escherichia coli* 119, *Pseudomonas fluorescence*, *Micrococcus luteus*, (causing asthma in human being) *Bacillus subtilis* and *Bacillus cerus* (cause food poisoning in human being)[10,11].

To explore the possible role of transitional metal ferrocyanides as antimicrobial agent against crop and human pathogens and other harmful microorganisms, the present studies have been undertaken to screen the complexes for their antibacterial activity.

EXPERIMENTAL

Collection of bacterial culture: Bacterial culture of *E. coli* 101, *E. coli* 119, *P. fluorescens*, *M. luteus*, *B. subtilis* and

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TABLE-1
BACTERIAL CULTURES USED IN PRESENT STUDY

Name of bacterial pathogens	Diseases caused	Place of collection	Optimum temperature (°C)
Escherichia coli 101	Non pathogenic	Department of Biotechnology and Bioscience, Banasthali University, Rajasthan, India	28 ± 1
Escherichia coli 119	Non pathogenic	Department of Biotechnology and Bioscience, Banasthali University, Rajasthan, India.	28 ± 1
Micrococcus luteus	Asthma	Department of Biotechnology and Bioscience, Banasthali University, Rajasthan, India	28 ± 1
Bacillus subtilis	Food poisoning	Department of Biotechnology and Bioscience, Banasthali University, Rajasthan, India	28 ± 1
Bacillus cereus	Food poisoning	Department of Biotechnology and Bioscience, Banasthali University, Rajasthan, India	28 ± 1
Pseudomonas fluorescens	Non pathogenic	Department of Biotechnology and Bioscience, Banasthali University, Rajasthan, India	28 ± 1

TABLE-2
ELEMENTAL ANALYSIS DATA OF METAL FERROCYANIDE COMPLEXES

Complexes	Found (calculated) (%)				
	M	Fe	N	C	H
$Mn_2[Fe(CN)_6]\cdot 3H_2O$	28.56 (29.23)	14.66 (14.86)	22.59 (22.36)	20.67 (19.17)	1.69 (1.61)
$Co_2[Fe(CN)_6]\cdot 2H_2O$	32.12 (32.22)	15.30 (15.27)	21.16 (22.97)	19.65 (19.70)	1.11 (1.10)
$Ni_2[Fe(CN)_6]\cdot 5H_2O$	27.85 (27.93)	13.00 (13.28)	18.79 (19.19)	16.51 (17.14)	2.22 (2.30)
$Cu_2[Fe(CN)_6]\cdot 7H_2O$	27.10 (27.32)	12.10 (12.01)	18.12 (18.07)	14.75 (15.49)	3.13 (3.03)
$Zn_2[Fe(CN)_6]\cdot 3H_2O$	32.84 (32.95)	14.10 (14.08)	20.40 (21.18)	17.74 (18.16)	1.51 (1.45)
$Cd_2[Fe(CN)_6]$	50.12 (51.47)	12.58 (12.79)	20.38 (19.24)	17.71 (16.50)	0.26 (0.00)

B. cerus were procured from Department of Biotechnology and Bioscience, Banasthali University, Rajasthan. They were cultured in Petri plates containing Nutrient Agar media and incubated at 28 ± 1 ºC. (Table-1)

Synthesis and characterization of metal ferocynides: Manganese, cobalt, nickel, copper, zinc and cadmium ferrocyanides were prepared following the Kourim's procedure[12]. A solution of potassium ferrocyanide (167 mL, 0.1 M) was added to solution of desired metal salt (500 mL, 0.1 M) with constant stirring at room temperature. A slight excess of metal salt solution markedly improves the coagulation of the precipitate. The reaction mixture was heated on a water bath at 80 ºC for 3-4 h and allowed to stand at ambient temperature for 24 h. The precipitate was filtered under vacuum and washed thoroughly with double distilled water. It was dried in an oven at 60 ºC. The dried product was ground and sieved to 100 mesh sizes. These complexes were characterized on the basis of elemental analysis, infrared spectra and magnetic susceptibility measurement (Tables 2-4).

Infra-red spectral data of Mn, Co, Ni, Cu, Zn and Cd ferrocyanides were recorded in KBr disc on Perkin Elmer FTIR spectrophotometer. IR spectra of the compounds show all the characteristic absorption frequencies for metal ferrocyanide complexes. A broad band in the range of 3750-3400 cm^{-1} in case of all metal ferrocyanides was observed due to interstitial water molecules and OH^- groups while the characteristic HOH bending appears at 1631-1600 cm^{-1} in case of all the complexes synthesized. A sharp peak at 2080 ± 10 cm^{-1} in case of all metal ferrocyanides is characteristic of cyanide stretching and sharp peaks at 691-590 cm^{-1} are characteristic of Fe-C stretching frequencies. The ν(M-N) was also observed in 508-451 cm^{-1} range (Table-3).

TABLE-3
INFRARED SPECTRAL DATA OF METAL FERROCYANIDE COMPLEXES

Complexes	Frequencies (cm^{-1})				
	ν_{HOH}	$\nu_{C\equiv N}$	HOH bending	ν_{Fe-C}	$\nu_{Metal-N}$
$Mn_2[Fe(CN)_6]\cdot 3H_2O$	3701	2070	1631	592	451
$Co_2[Fe(CN)_6]\cdot 2H_2O$	3724	2083	1609	592	465
$Ni_2[Fe(CN)_6]\cdot 5H_2O$	3697	2091	1611	592	463
$Cu_2[Fe(CN)_6]\cdot 7H_2O$	3845	2090	1621	592	503
$Zn_2[Fe(CN)_6]\cdot 3H_2O$	3685	2080	1600	603	496
$Cd_2[Fe(CN)_6]$	3724	2071	1623	590	508

TABLE-4
MAGNETIC MOMENTS AND MOLAR CONDUCTIVITY OF METAL FERROCYANIDE COMPLEXES

Metal hexacyano-ferrate (II)	μ_{calc} (B.M.)	μ_{eff} (B.M.)	Molar conductance (µS)
$Mn_2[Fe(CN)_6]\cdot 3H_2O$	5.92	6.21	24.2
$Co_2[Fe(CN)_6]\cdot 2H_2O$	3.87	4.36	9.81
$Ni_2[Fe(CN)_6]\cdot 5H_2O$	2.83	2.99	6.61
$Cu_2[Fe(CN)_6]\cdot 7H_2O$	1.73	2.45	6.72
$Zn_2[Fe(CN)_6]\cdot 3H_2O$	0.00	0.81	2.70
$Cd_2[Fe(CN)_6]$	0.00	0.90	7.44

All the synthesized metal ferrocyanides are expected to be diamagnetic due to paired electrons. However, the outer cations may contribute to observed magnetic moment, if any. Observed magnetic moment values (Table-4) of these metal hexacyanoferrates were found to be in good agreement with calculated values. μ_{obs} indicate presence of three unpaired electrons in cobalt ferrocyanide, which is in agreement with d^7 configuration of Co^{2+}, whereas reported value of magnetic moment for cobalt ferrocyanide is 4.6 BM. μ_{obs} values revealed that five, three, two and one unpaired electrons are present in Mn(II), Co(II), Ni(II) and Cu(II) hexacyanoferrates respectively, while zinc and cadmium hexacyanoferrates have zero magnetic moments. On the basis of conductivity measurements (Table-4) in non-aqueous solvent (DMSO), it can be concluded that they are non-electrolytes.

TABLE-5
ANTIBACTERIAL ACTIVITY OF TRANSITION METAL FERROCYANIDES

Name of metal complexes	Inhibition zone (mm) against bacterial strains					
	E. coli 101	*E. coli* 119	*B. subtilis*	*B. cerus*	*M. luteus*	*P. fluorescence*
Cadmium ferrocyanide	6	0	6	0	19	25
Cobalt ferrocyanide	0	6	18	0	0	12
Copper ferrocyanide	13	16	5	18	14	2
Zinc ferrocyanide	18	0	0	0	23	0
Nickel ferro cyanide	0	0	0	0	0	0
Magnese ferrocyanide	7	6	0	0	10	5

On the bases of elemental analysis data, magnetic susceptibility measurements, thermo gravimetric and differential thermal analysis Zn(II), Co(II), Cu(II), Cr(III), Ni(II), Mn(II) and Cd(II) ferrocyanides were characterized. From a structural stand point, the ferrocyanide ion can be considered to be a good example of strong field (low spin) octahedral complexes. In the presence of the strongly perturbing cyanide ligand the 3d orbitals of ferrous ion will get splitted, causing a relatively large separation between t_{2g} and eg orbitals. In the ground state, therefore, the six electrons from Fe(II) ion will be placed in the low lying t_{2g} orbitals. The metal ions like Zn^{2+}, Co^{2+}, Cu^{2+}, Cr^{3+}, Ni^{2+}, Mn^{2+} and Cd^{3+} will remain in the lattice.

The molecular formula of synthesized metal complexes established on the basis of data obtained from elemental analysis have been established as $Mn_2[Fe(CN)_6]\cdot3H_2O$, $Co_2[Fe(CN)_6]\cdot2H_2O$, $Ni_2[Fe(CN)_6]\cdot5H_2O$, $Cu_2[Fe(CN)_6]\cdot7H_2O$, $Zn_2[Fe(CN)_6]\cdot3H_2O$ and $Cd_2[Fe(CN)_6]$ respectively.

Screening of metal complexes for antibacterial activity

Disc diffusion method: Screenings of metal ferrocyanides for antibacterial activity have been done by the disc diffusion method[13]. The antibacterial activity have been assessed using the simple disc diffusion method where metal ferrocyanides impregnated filter paper disc are placed on nutrient agar media incubated with the test bacteria so as to get a lawn culture on incubation. The complex diffuses in to the medium and inhibits bacterial growth around the disc. Fifty micro liters of the test bacterial culture were spread over the plates containing nutrient agar. Sterile paper discs of 0.5 cm diameter were loaded in condensed aqueous solvent and suspension of metal complexes respectively. These were dried and placed on solid media incubated with test bacteria. Two discs loaded with complex and other two loaded with similar amount of solvent for control were placed on petri-dishes containing bacterial culture. The plates were left for 30 min. at room temperature to allow the diffusion of the metal ferrocyanides and then they were incubated at 28 ºC, inhibition zone have been measured after 48 h of incubation. After the incubation period, the zone of inhibition was measured with a ruler. For screening of antibacterial activity of metal ferrocynide by paper disc method 0.03 mg metal complexes per disc was loaded. Studies were performed in duplicate, the mean value was calculated.

RESULTS AND DISCUSSION

Six transition metal ferrocyanides have been screened for biocidal activity against six bacterial species namely *Escherichia coli* 101, *Escherichia coli* 119, *Pseudomonas fluorescence*, *Micrococcus luteus, Bacillus subtilis* and *Bacillus cereus*. The results are reported in Table-5. Cobalt ferrocyanide has shown maximum bactericidal potential against *B. subtilus* while cadmium and copper ferocyanide have shown maximum activity against *P. florescence* and *B. cerus* respectively.

Copper ferrocyanide has been found to possess significant activity mm against all the test pathogens. Zinc ferrocyanide has ranging from 2-8 significant activity against *E. coli* 101 and *M. luteus*. Magnese ferrocyanide also possesses significant antibacterial potential against *E. coli* 101, *E. coli* 119, *M. luteus* and *P. fluorescence*. Nickel ferrocyanide has not exhibited activity against any of the test pathogens.

Conclusion

There are few reports on synergistic effect of antimicrobial activity of metal ferrocyanide with botanicals[13]. These complexes have also been reported to adsorb biomolecules. There may be the possibility of adsorption of active ingredient at the surface transitional metal ferrocyanides. Thus concentration and shelf life of other active agents may increase and may result in increased activity (synergistic effect). Such studies may be helpful in development of antibacterial formulations against harmful microorganisms.

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