International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2015): 6.391

Analytical and Antibacterial Studies of Mn (II), Co (II) and Ni (II) Complexes

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Abstract: ***The ligands possess anti-bacterial, anti-fungal, anti-inflammatory, anti-diabetic, anti-tumor, anti-proliferative, anti-cancer, anti-malarial, anti- inflammatory, herbicidal, medicinal properties. The chelating ligands act as more active agents than non-chelating ligands. Benzaldehyde and substituted benzaldehydes react with hydroxyl amine, semicarbazide, hydrazine etc. to give derivatives like oximes, semicarbazones, and hydrazones which act as good chelating ligands. These derivatives have long been prepared and analyzed by chemists for anti-bacterial, anti-fungal, anti-tubercular, anti-inflammatory, anti-diabetic, anti-tumor, anti-proliferative, anti-cancer, anti-malarial and analgesic activities. The ligands which are active against micro-organisms like bacteria, fungi, or virus are finding importance in pharmaceutical chemistry. When the chelating ligands are complexed with the metal ions their activities may alter. In view of these facts here we have undertaken the screening of metal complexes formed by combination of metals Mn (II), Co (II) and Ni (II) with ligands 2-pyrazinoyl hydrazide and its derivatives with various aromatic and heterocyclic aldehydes against various bacteria. The present paper deals with the characterization and screening of metal-complexes of hydrazones derivatives of 2-pyrazinoyl hydrazide with various aromatic and heterocyclic aldehydes such as benzaldehyde, anisaldehyde, 4-hydroxy-3-methoxy benzaldehyde, p- (N,N-diethyl amino) benzaldehyde, cinnamaldehyde, 4-methyl salicyldehyde and 2-furfuraldehyde against some gram (+ve) and gram (–ve) bacteria.***

Keywords: Antibacterial, Substituted aldehydes, Ligands, Metal-complexes, Hydrazones.

1. Introduction

The metal-ligand complexes play important role in a number of biological processes and biological systems. These observations have led the researchers from different fields [1-3] to pay attention to this interesting field. As such metal-ligand complexes show large number of biological and medicinal activities viz. Anti-bacterial, anti-fungal [4-7], anti-inflammatory activities [8-13], anti-diabetic [14], anti-tumor [15-16], anti-proliferative [17-18], anti-cancer, herbicidal [19], anti-corrosion etc. At the same time these can act as catalysts for many reactions [20-22]. The five or six membered-chelates rings formed by chelating ligands and metal ions are able to produce the metal containing cross linking agents with required properties [23] .It is well known that both N and S atoms play a key role in the coordination of metals at the active sites of numerous metallobiomolecules [24] . Metallorganic chemistry has emerged as an important field of research due to the demand of new metal based antibacterial and antifungal compounds [25-26] . The alteration in antibacterial properties of metal complexes, as compared to that of free metal and free ligand may be correlated to one of the following facts.

(a) By an increase in the liposoluble nature of the biologically active ligand or metal ion in the form of metal complex in comparison to the metal ion or the ligand molecule alone.
(b) By the replacement of the metal ion of the metal enzyme present in biological system with the foreign metal ion of the more liposoluble metal complex. This situation may arise only when foreign metal ion of the more liposoluble metal complexes has a stronger chelating tendency to attach with the protein residue on the metal enzyme.
(c) By the displacement of protein molecule from the metal enzyme by the foreign ligand of the more liposoluble metal ligand complex, thus rupturing the enzyme affecting a biological system. This is possible only when the foreign ligand is capable of being coordinated to the metal ion of the metal enzyme more strongly than the protein molecule in the system.
(d) By an increased activity of a complex as a whole not depending on its constituting metal and the ligands.
(e) Apart from the above factors, a comparative faster, diffusion of the metal complex as a whole through the cells of fungi and bacteria may be of one of the important factors. It is evident that their complexes are stable. Such compound can exert a powerful inhibitory effect on an intracellular biological process by concentrating at susceptible site from which it dissolute slowly .

Considering these facts the Schiff base ligands 2-pyrazinoyl hydrazones were synthesized by reacting 2-pyrazinoyl hydrazide with benzaldehyde and substituted benzaldehydes (1:1) [27] and a series of metal complexes with these ligands were synthesized by reaction with Mn (II), Co (II) and Ni (II) metal salt. The Schiff base ligands and their complexes have been characterized[28] with the help of elemental analysis, conductance measurements, magnetic measurements and their structure configuration have been determined by various spectroscopic (Electronic, IR, 1H NMR) techniques. Electronic and magnetic moments of the complexes indicate that the geometries of the metal centers were octahedral [28] .

Considerable interest has been shown in the study of the complexes of transition metal ions with various aromatic and heterocyclic derivatives of pyrazine-2-carboxylic acid hydrazide. It is mainly due to potentially multidentate ligational behaviour of these pyrazine-2-carboxylic acid hydrazides. More recently interest has been growing in the synthesis and studies of their chemotherapeutical behavior particularly their antibacterial activity.

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2. Materials and Method

The ligands 2-pyrazinoyl hydrazones were synthesized by treating ethanolic solutions of 2-pyrazinoyl hydrazide with benzaldehyde and substituted benzaldehydes (1:1) [27] .They were characterized with the help of elemental analysis, conductance measurements and magnetic measurements.

These ligands i. e. Pyrazine-2-carboxylic acid hydrazide (PAH;$C_5H_6N_4O$) and its hydrazones derivatives with different aldehydes viz. Benzy1lidene-2-Pyrazinoyl hydrazone (PAH- BENZ ;$C_{12}H_{10}N_4O$), Anisalidene-2-pyrazinoyl hydrazone (PAH- ANSL ;$C_{13}H_{12}N_4O_2$;) 4-Hydroxy-3-methoxy benzylidene-2-pyrazinoyl hydrazone (PAH-VANI;$C_{13}H_{12}N_4O_3$), p - (N, N - diethyl amino) benzy1lidene–2-pyrazinoyl hydrazone (PAH-PDEAB ;$C_{16}H_{19}N_5O$), Cinnamalidene-2-pyrazinoyl hydrazone (PAH-CAH; $C_{14}H_{12}N_4O$), 4-Methyl salicylidene-2- pyrazinoyl hydrazone (PAH- MSALI ;$C_{13}H_{12}N_4O_2$), 2- Furfuralidene-2'- pyrazinoyl hydrazone (PAH- FURAL ;$C_{11}H_{10}N_4O_2$) were treated with metal salts of Mn(II), Co(II) and Ni(II) to synthesize complexes. These complexes have been characterized [28] . Further they have been screened for their antibacterial activity against some human pathogen.

(I) Brief Description of Test Organisms viz. Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Bacillus pumilus and Bacillus subtilis:

(a) **Escherichia coli:** It inhabits human and animal intestinal tract. [29] These organisms are gram (-ve), non-sporing, occasionally encapsulated, variably motile bacilli, 0.5 to 0.7µm in width and 1 to 4µm in length, occur either singly, in pair, or in long chains. They sometime occur in oval or coccal shapes and occasionally as very long filaments, they vary from colourless to grayish white. E. coli is aerobic or facultatively anaerobic and is easily grown on common laboratory media. Although optimum growth occurs at 37°C, growth is also obtained over a temperature range of 10°C to 46°C. The resistance of E. coli to heat, in average for only a few strains survives 60°C for 30 minutes. E. coli is not only very pathogenic for either men or animals, but it is a major source of vitamin K [30] and in some persons a secondary source [31-32] of vitamin B2. Infection with E. coli occurs occasionally in the appendix and gall bladder wounds.

(b) Staphylococcus aureus: The staphylococci are a group of gram (+ve), spherical bacteria that form irregular cellular aggregation or group like clusters but seldom forms chains when grown in liquid medium. The pathogenic staphylococci grow abundantly at temperature ranging from 35-37°C. They grow best on a slightly alkaline medium (pH 7.40). They are resistant to heat (60°C for 30 minutes). They are non-sporing, non-motile, facultatively anaerobic.
S. aureus is a parasitic and pathogenic. [33-34] The staphylococcus causes a wide variety of diseases in man. S. aureus is a common inhabitant of human skin, throat [35] and mucous membrane. It is also a pathogenic bacterium of milk. Bovine mastitis is an inflammatory and highly communicable disease caused by S. aureus. The most common type of food poisoning is generally referred as staphylococcus or staph food poisoning. [36]

(c)Klebsiella pneumoniae: Kiepsiella pneumoniae called friend landor's bacilli was first isolated from the lung of a patient dying with pneumonia, is a normal inhabitant of nasal, oral cavities and the intestinal tract.[37] It is involved in respiratory and some supportive infections. [38]It is a gram (-ve),non-sporing, non-motile, facultatively anaerobic rod, 0.3 to 0.5 µm in width by 2 to 5 µm in length, growth occurs on usual agar media between 15°C to 40°C, with an optimum temperature of 37°C. K. Pneumoniae is present in less than 5% of all normal human respiratory tracts. Pneumonia by K. pneumoniae is highly fatal.

(d)Bacillus pumilus: It is rod-like in shape, with zig-zag twisting, 0.2 to 2.5 µm by 1.32 to 7.2 µm.Gram (+ve), found in moist soil [39] and sewage.

(e)**Bacillus subtilis:** It is rod-shaped, 0.3 to 2.2 µm by 1.27 to 7.0 µm. Majority motile flagella typically lateral. Endospores formed, not more than one in a sporangial cell, gram (+ve), chemoorganotrophs. Commonly found in soil. [39]

Table 1: NCBI No, ATCC No and NCTC No. of test organisms used in experiment are as follows.

	NCBI No.	ATCC No.	NCTC No.
(1) Escherichia coli	----------	10536	----------
(2) Staphylococcus aureus	----------	29737	----------
(3) Klebsiella pneumonia	9111	10031	----------
(4) Bacillus pumilus	----------	14884	8241
(5) Bacillus subtilis	----------	6633	8236

(II) Techniques Used for Bacteriological Studies

The technique involves the sterilization of apparatus and measurement of bacteriostatic action. Out of a number of known methods [40] the cup plate method has been employed. After taking suitable nutrient media experimental broths were prepared. The measured quantities of the cultures of the test organisms (0.2 ml per 100 ml) were added to heated medium (nearly 45-50°C) and the inoculated media was immediately poured into the sterilized Petri dishes to give a uniform depth of 3 to 4 mm and then allowed to set on an even surface. There after it was transferred to a refrigerator maintained at 5 ± 2°C (to minimize the effect of variation in time between the applications of the different solution). The prepared plates were stored in such a manner so that no significant growth or death of the test organisms occur before dishes are used and that the surface of the agar layer is dry at the time of use.

Table 2: Incubation temperature of test organisms (Bacteria)

S.No.	Bacteria	Incubation temperature (°C)
1.	Escherichia coli	35.40
2.	Staphylococcus aureus	32.00
3.	Klepsiella pneumoniae	35.40
4.	Bacillus pumilus	38.00
5.	Bacillus subtilis	32.00

The test solutions of metal complexes were prepared (1.0 mg/ml) in dimethyl sulphoxide (DMSO).Then 0.2 ml of each solution was poured into the cups made by a sterilized

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cutter. All Petri dishes were again put in the refrigerator for at least one hour in order to allow diffusion of the solution and then transferred to incubator maintained at specified temperature (Table 2.) for about eighteen hours. Consecutively three tests were carried and the average zones of inhibition were noted. The zone of inhibitions around the holes, were measured using Vernier Caliper and data was summarized in the table (4a, b and c.).The activities are represented by diameter of zone of inhibition (mm).

3. Results and Discussion

Infra red Spectral Studies

In comparison to the Infrared spectra of free ligands, the Infrared spectra of complexes of Mn(II), Co(II) and Ni(II) with ligands pyrazine-2-carboxylic acid hydrazide ($C_5H_6N_4O$); benzylidene-2-pyrazinoyl hydrazone ($C_{12}H_{10}N_4O$); anisalidene-2-pyrazinoyl hydrazone ($C_{13}H_{12}N_4O_2$); 4-hydroxy-3-rnethoxy benzylidene-2-pyrazinoyl hydrazone ($C_{13}H_{12}N_4O_3$); N,N-Diethyl amino benzylidene-2-pyrazinoyl hydrazone ($C_{16}H_{19}N_5O$); cinnamalidene-2-pyrazinoyl hydrazone ($C_{14}H_{12}N_4O$); 4-methyl salicylicdene-2-pyrazinoyl hydrazone ($C_{13}H_{12}N_40_2$) and 2-furfuralidene-2'-pyrazinoyl hydrazone ($C_{11}H_{10}N_4O_2$) (data summarized in table 3a, b and c) show some variations. The absorption band at 3500 cm^{-1} and 3450 cm^{-1} observed in case of (PAH-VANI;$C_{13}H_{12}N_4O_3$) and (PAH- MSALI ;$C_{13}H_{12}N_4O_2$) respectively tentatively assigned to ν(OH) attached to the phenyl ring in the free ligand.(PAH- MSALI ;$C_{13}H_{12}N_4O_2$) shows a characteristic decrease in intensity as well as in the frequency nearly 40 cm^{-1} in the IR spectra of the complexes, showing thereby participation of phenolic group in chelation.

The general observations in shifts of band positions in the spectra of complexes are,

1) The ν (NH) of the ligand at 3200 cm^{-1} do not shift or disappeared in the spectra of all the complexes suggesting non participation of ν NH. [41]
2) The amide band I (νC=O) in the complexes was shifted to lower wave numbers (Δν = 30 cm^{-1}) indicating the involvement of carbonyl oxygen in bond formation.
3) The position of the amide bands having contribution from ν (C=N), δ(N-H) in the region 1500 to 1000 cm^{-1} either did not shifted or the shifts were too small to draw any definite conclusions from the direction of shifts.
4) The ligands exhibit the ν (C=N) in the region 1605-1645 cm^{-1} and the band shifts to lower energy side in complexes indicating nitrogen coordination.
5) A shift of the ν(C-O) (phenolic) of the ligand (~1540 cm^{-1}) to higher energy side by ~20 cm^{-1} on coordination constitutes an unambiguous evidence [42-44] for the formation of phenolic oxygen bond (PAH- MSALI; $C_{13}H_{12}N_4O_2$).
6) The strong to medium intensity bands observed at 3330 and 3090 cm^{-1} in the spectrum of ligands are assigned to ν_{as} NH and ν_s NH vibrations respectively [45] of secondary amines group. The bands due to ν (NH) remain unaltered or show slight blue shifts in all the complexes of ligands suggesting no coordination through secondary amino group of the hydrazine residue.
7) The medium to strong bands observed in the region 3270-3200 and 3350-3170 cm^{-1} are attributed to NH_2 stretch of the amino[46] group in ($C_5H_6N_4O$). The bands due to ν (NH) remain unaltered or show slight blue shifts in all the complexes of ligand suggesting no coordination through secondary amino group of the hydrazine residue.
8) The NH_2 stretching frequencies of the primary amino group in the complexes, however shift to lower frequencies side with loss in intensity. This is an indication of coordition of amino group through nitrogen in the complexes of the ligand ($C_5H_6N_4O$). The coordination of amino group of hydrazine residue of ligand ($C_5H_6N_4O$) is further supported by the blue shift of (N-N) stretch [47] from 940 to 945-950 cm^{-1}. Likewise the coordination of amino group of ligand is supported by positive shift of (C-N) stretch from the frequency range 1385-1340 cm^{-1} to 1390-1350 cm^{-1}.
9) The strong to medium intense bands observed in the region 1210-1140 cm^{-1} in the spectra of free ligand ($C_{11}H_{10}N_4O_2$) is ascribed to (C-O-C) stretch of the furan ring. On complex formation these shift to lower frequency side with varying intensities suggest the involvement of furan oxygen in coordination in all complexes. The involvement of furan oxygen in coordination is further supported by the appearance of non ligand bands in the region 560-500 cm^{-1} in the far IR-spectra of the complexes [48].

Far Infrared spectra (600-200cm^{-1}): The spectra of the complexes in the region 600-200 cm^{-1} exhibit some characteristic and medium intensity bands. Brown and Kubota [49] reported different regions of ν (M-Cl) depending upon the stereochemistry. In octahedral and tetrahedral complexes ν (M-Cl) are reported by various workers [50] in the range 250-220cm^{-1} and 350-280 cm^{-1} respectively. It is reported [51] that in the complexes having one coordinated halogen, the stretching frequencies ν (M-Cl) are intermediate between those found in tetrahedral and octahedral complexes.

The medium to strong bands observed in the region 415-380 cm^{-1} in the complex are attributed to (M-N) stretching vibrations. The ν (M-Cl) stretching bands are attributed in the region 350-275 cm^{-1} in all the complexes [52] .

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Table 3(a): Selected I.R Frequencies (cm^{-1}) and their tentative assignment from I.R. spectra of manganese (II) complexes with pyrazoline-2- carboxylic acid hydrazide and its derivative

S. No.	Compound	Amide band I ν (C=O)	Amide band IIν(-CH=N-) of immine-N & NH bending modes.	Antisymmetric & symmetric ν (C=C)+ ν (C=N) of pyrazine ring.	Amide band III ν (C=O) + ν (C=N)+ γ(CO) + γ (CN)	Amide band IV γ (NCO) γ (C-O)	Pyrazine ring breathings, deformation δ(N-N)*	Metal donor frequencies 1. ν (M-azomethine- N) 2.ν(M-enolic-O) 3. ν (M-halogen)
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1.	$[Mn(C_{13}H_{12}N_4O_3)_2CL_2]$	1630vs 1600sh	1570vs 1560sh	1520vs	1410ms 1325ms	1210ms 1185s 1140ms	1065ms 1025ms 900vs*	1. 440ms 2. 330 s 3. 265 ms
2.	$[Mn(C_{16}H_{19}N_5O)_2CL_2]$	1640vs 1610sh	1580vs	1525s	1380ms	1205vs 1165s	1060ms 1025ms 910vs*	1. 478ms 2. 336 s 3. 282 ms
3.	$[Mn(C_{14}H_{12}N_4O)_2Cl_2]$	1610vs 1600sh	1580ms	1560sh	1385vs 1330ms	1200s 1165ms	1065ms 1025ms 895vs*	1. 470ms 2. 335 s 3. 280 ms

S-sharp ms-medium sharp vs-very sharp sh-shoulder b-broad vb-very broad

Table 3 (b): Selected I.R Frequencies (cm^{-1}) and their tentative assignment from I.R. spectra of cobalt (II) complex with pyrazine-2- carboxylic acid hydrazide and its derivative

S. No.	Compound	Amide band I ν (C=O)	Amide band II ν(-CH=N-) of immine-N & NH bending modes.	Antisymmetric & symmetric ν (C=C)+ ν (C=N) of pyrazine ring.	Amide band III ν (C=O) + ν (C=O)+ γ(CO) + γ (CN)	Amide band IV γ (NCO) γ (C-O)	Pyrazine ring breathings, deformation δ(N-N)*	Metal donor frequencies 1. ν (M-azomethine- N) 2. ν (M-enolic-O) 3. ν (M-halogen)
1	2	3	4	5	6	7	8	9
1.	$[Co\ (C_5H_6N_4O)_2Cl_2]$	1650vs 1610vs	1580s	1570sh 1540s	1360vs	1140ms	1030vs 880vs*	1. 470m 2. 320m 3. 280s
2.	$[Co(C_{12}H_{10}N_4O_2)_2Cl_2]$	1615s	1585sh 1560ms	1540ms	1380ms 1365vs	1240ms 1200ms 1160ms	1090b 1035vs 875s*	1. 475ms 2. 335m, 330ms 3. 280 ms
3.	$[Co\ (C_{13}H_{12}N_4O)_2Cl_2]$	1610vs	1580vs	1545s 1520ms	1380s 1360sh 1330vs	1210ms 1185vs 1130s	1065vs 1040vs 885vs*	1. 475ms 2. 328ms,325s 3. 282 ms
4.	$[Co\ (C_{13}H_{12}N_4O)_3Cl_2]$	16050sh	1570vs 1560sh	1505vs	1360sh 1405vs 1320ms	1220ms 1185ms 1150ms	1080b 1025vs 900vs*	1. 475m 2. 325m 3. 280 m
5.	$[Co\ (C_{16}H_{19}N_5O)_2Cl_2]$	1570vs	1570vs	1525ms	131380ms	1210ms 1175s 1150s	1055s 1020s 910s*	1. 470m 2. 330m 3. 280m
6.	$[Co\ (C_{14}H_{12}N_4O)_2Cl_2]$	1620vs 1600vs	1570s	1555s	1380vs 1325ms	1200s 1165s 1145ms	1055ms 1025vs 895vs*	1. 465m 2. 330m 3. 275s
7.	$[Co\ (C_{13}H_{11}N_4O_2)_2Cl_2]$	1600s	1600s	1580b	1380ms 1360sh 1325ms	1190s 1150b	1070s 1020vs 885vs*	1. 460ms 2. 340m 3. 270 ms
8.	$[Co\ (C_{11}H_{10}N_4O)_2Cl_2]$	1630vs 1610sh	1590vs	1550vb	1420ms 1360s	1210s 1180ms 1140s	1060vs 1020vs 900ms*	1. 465ms 2. 340ms 3. 280 ms

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S-sharp ms-medium sharp vs-very sharp sh-shoulder b-broad vb-very broad

Table 3 (c): Selected I.R Frequencies (cm^{-1}) and their tentative assignment from I.R. spectra of nickel (II) complexes with pyrazine-2- carboxylic acid hydrazide and its derivative

S. No.	Compound	Amide band I ν (C=O)	Amide band II ν (-CH=N-) of immine- N &NH bending modes.	Antisymmetric & symmetric ν (C=C)+ ν (C=N) of pyrazine ring.	Amide band III ν (C=O) + ν (C+O)+ γ(O) + γ (CN)	Amide band IV γ (NCO) γ (C-O)	Pyrazine ring breathings, deformation δ(N-N)*	Metal donor frequencies 1. ν (M-azomethine- N) 2. ν (M-enolic-O 3. ν (M-halogen)
1	2	3	4	5	6	7	8	9
1.	[Ni $(C_5H_6N_4O)_2Cl_2$]	1640vs 1600vs	1580ms	1565sh 1540s	1370ms	1130vs	1030vs 885vs*	1. 480ms 2. 330m 3. 280m
2.	[Ni $(C_{12}H_{10}N_4O)_2Cl_2$]	1610vs	1590vs 1575ms	1550ms	1400ms 1360vs	1200vs 1175ms 1100vs	1065ms 1030s 885s*	1. 470m 2. 340m 3. 285s
3.	[Ni $(C_{13}H_{12}N_4O_2)_2Cl_2$]	1610vs	1580vs	1555ms 1515s	1400ms 1360sh 1335ms	1205vs 1180ms 1130s	1070vs 1045ms 890vs*	1. 475m 2. 330m 3. 280s
4.	[Ni $(C_{13}H_{12}N_4O_3)_2Cl_2$]	1600mh 1600sh	1575ms	1560sh 1510vs	1420vs 1335ms	1215ms 1180vs 1145ms	1060vs 1015ms 900vs*	1. 470m 2. 330m 3. 270s
5.	[Ni $(C_{16}H_{19}N_5O)_2Cl_2$]	1620vs 1600sh	1570vs	1520s	1390ms	1210ms 1165s 1140s	1050ms 1025s 905s*	1. 475ms 2.340ms,330s 3. 280ms
6.	[Ni $(C_{14}H_{12}N_4O)_2Cl_2$]	1620vs 1590sh	1610sh 1580ms	1560ms	1400vs 1340ms	1200ms 1165s	1065vs 1025vs 900vs*	1. 475ms 2. 335ms, 325s 3. 280s

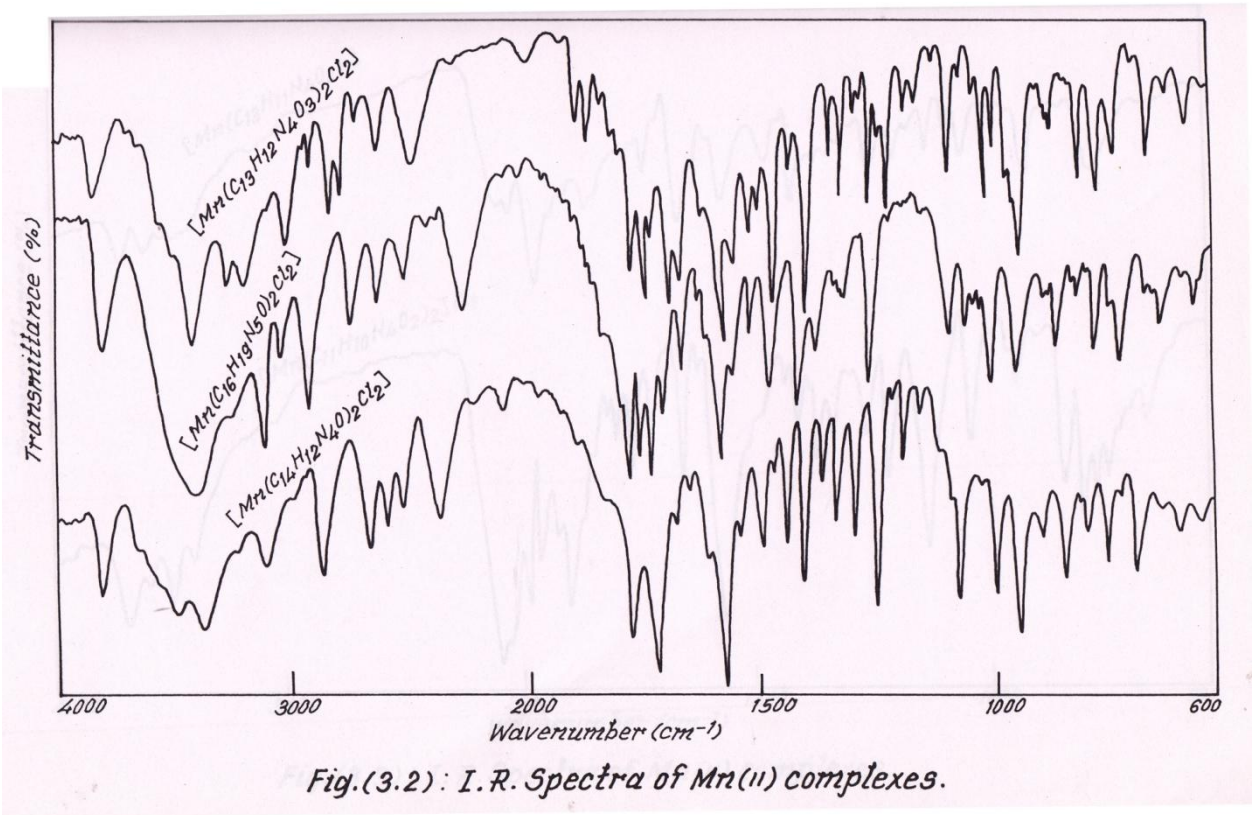


Fig.(3.2) : I.R. Spectra of Mn(II) complexes.

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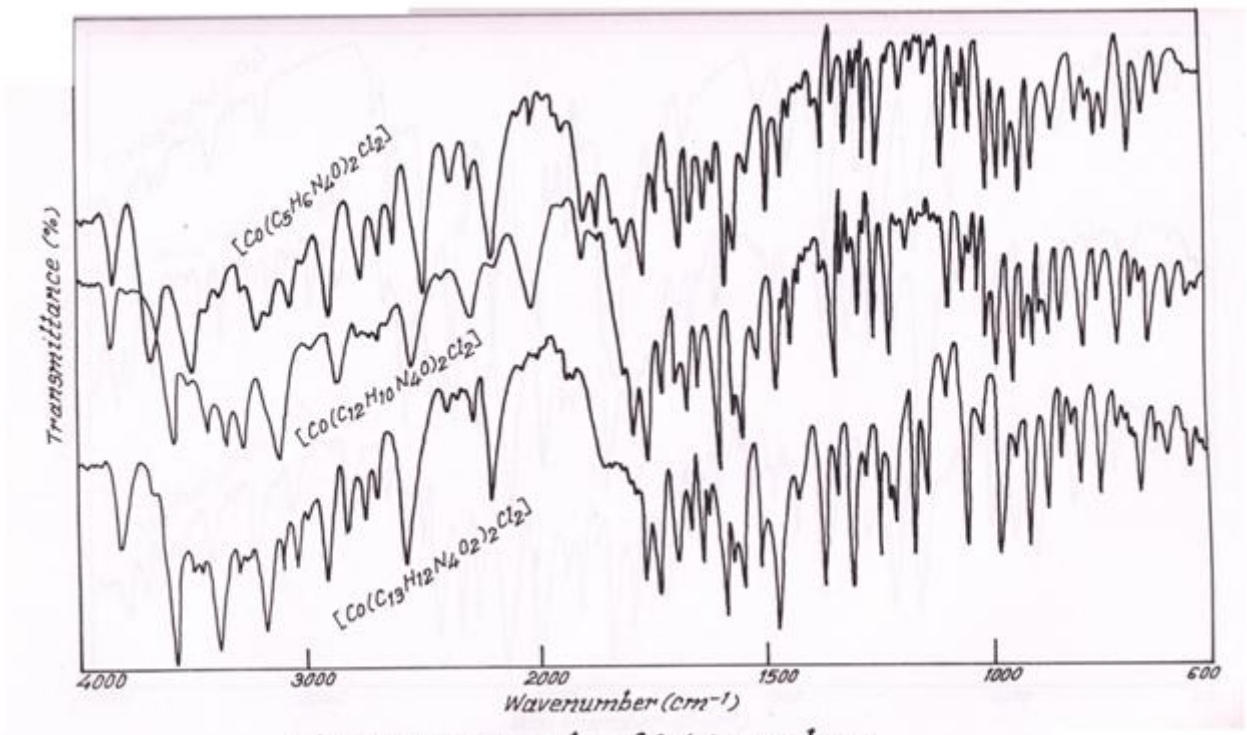


Fig.(3.4) : I.R. Spectra of Co(II) complexes.

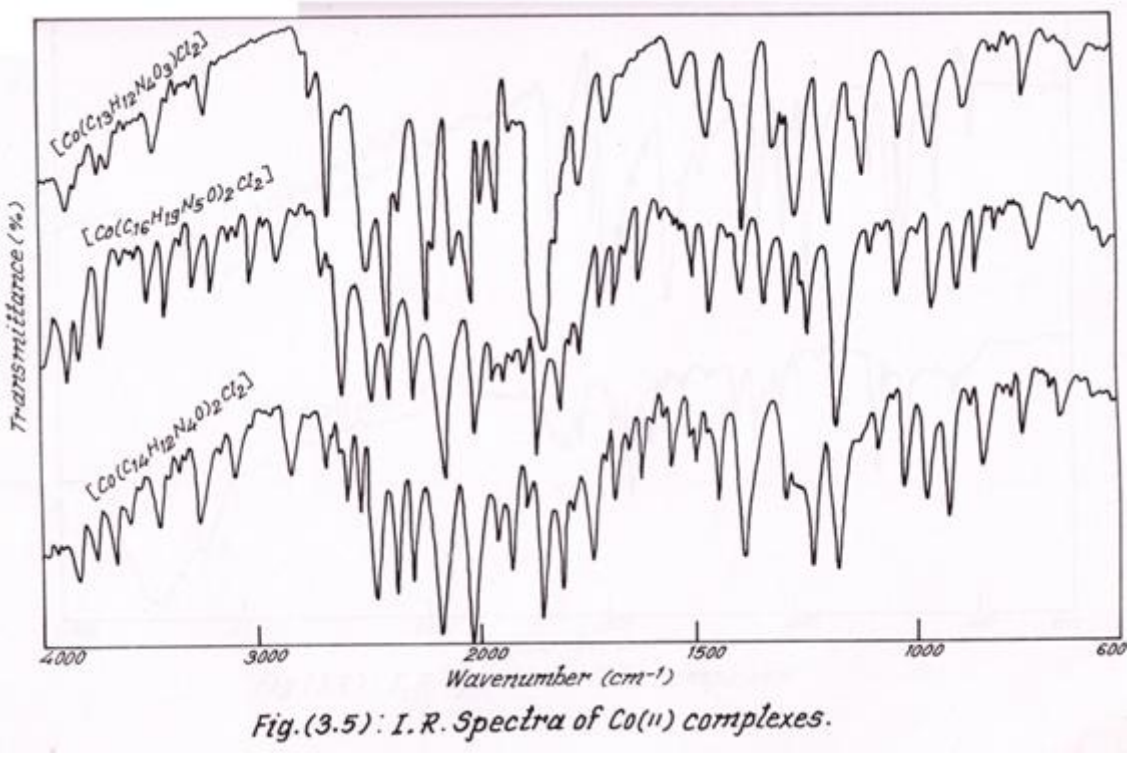


Fig.(3.5) : I.R. Spectra of Co(II) complexes.

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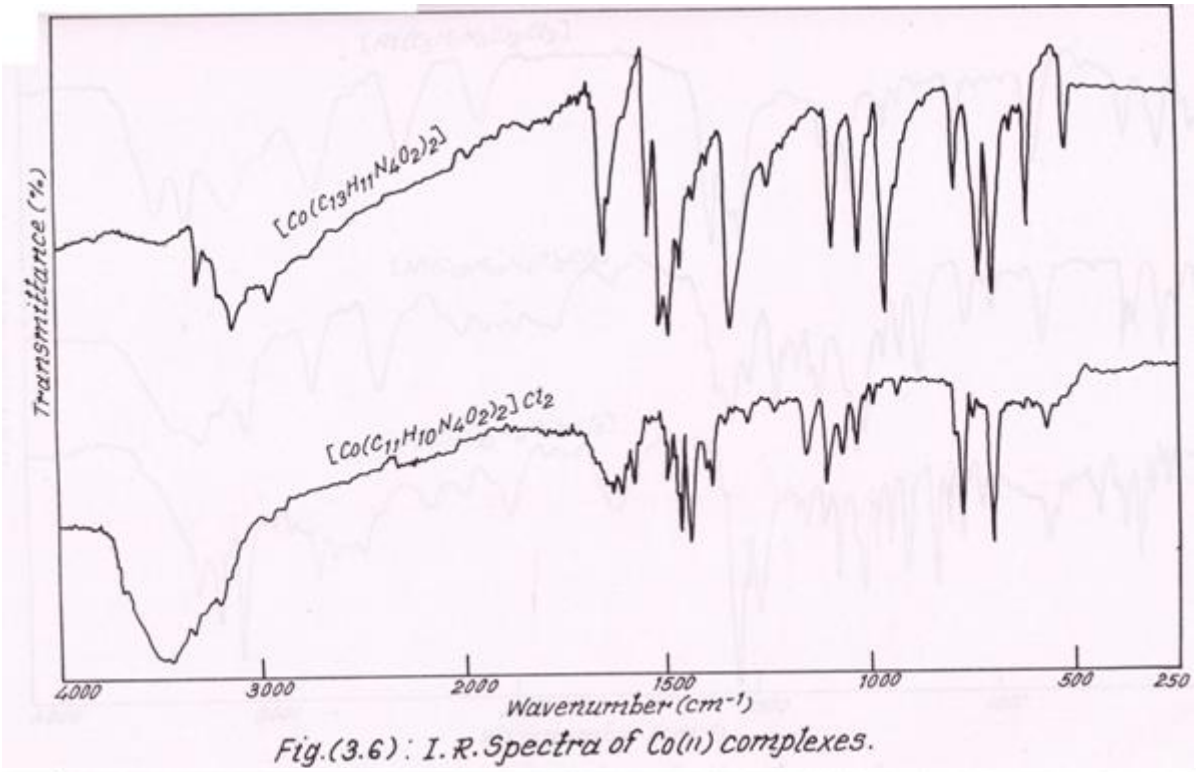


Fig.(3.6): I.R. Spectra of Co(II) complexes.

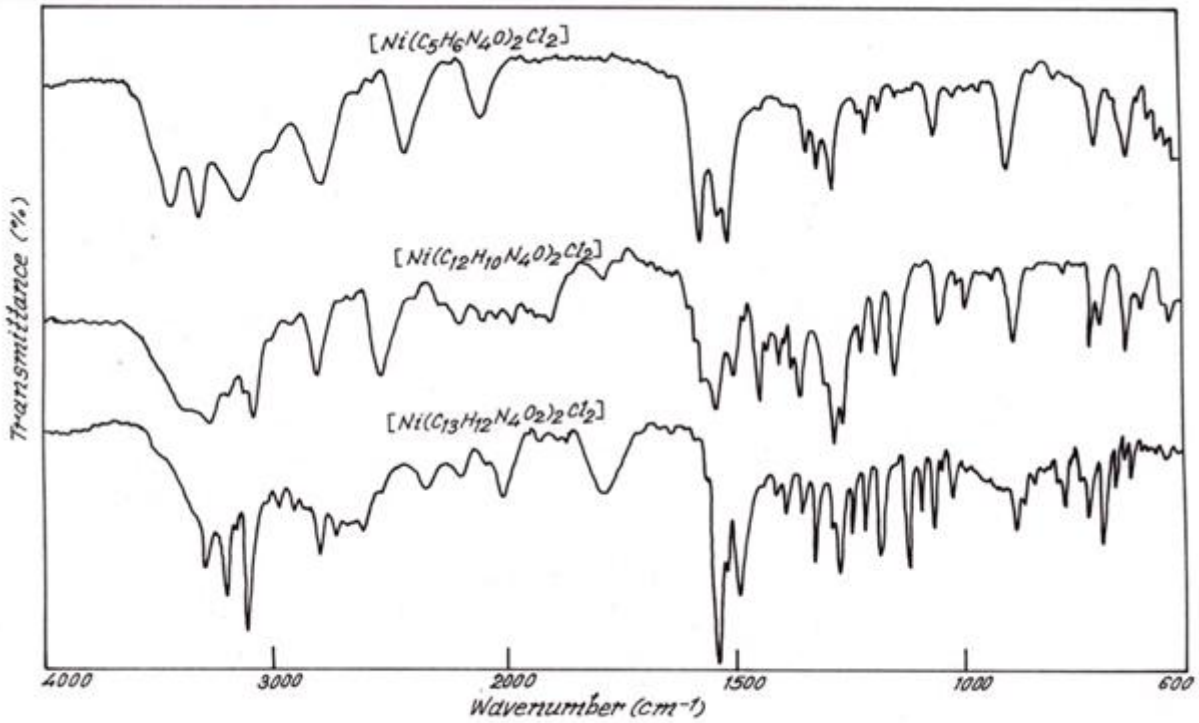


Fig.(3.7): I.R. Spectra of Ni(II) complexes.

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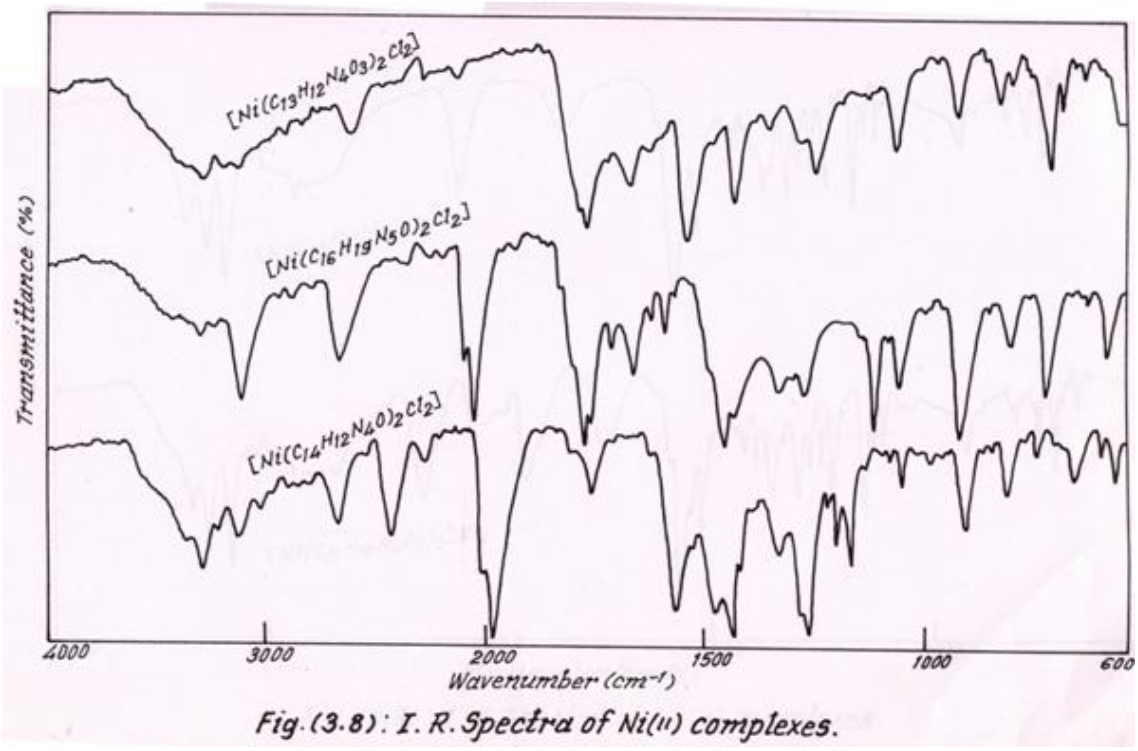


Fig. (3.8): I. R. Spectra of Ni(II) complexes.

Table 4a: Antibacterial activity of Manganese (II) complexes of ligands pyrazine-2-carboxylic acid hydrazide and its various derivatives against gram positive and gram negative organisms

S. No.	Compound	Gram Positive Organisms			Gram Negative Organism	
		S.aureus	B.subtilis	B.pumilus	E.coli	K.pneumoniae
		Diameter of Zone of Inhibition (mm)	Diameter of Zone of Inhibition (mm)	Diameter of Zone of Inhibition (mm)	Diameter of Zone of Inhibition (mm)	Diameter of Zone of Inhibition (mm)
1.	$[Mn(C_{13}H_{12}N_4O_3)_2Cl_2]$	7.8	7.8	7.8	14.1	16.5
2.	$[Mn(C_{16}H_{19}N_5O)_2Cl_2]$	15.2	7.8	7.8	14.2	7.8
3.	$[Mn(C_{14}H_{12}N_4O)_2Cl_2]$	16.0	7.8	11.8	14.1	7.8

(1) Zones of inhibition (mm) are the mean of three experimental observations.

Table 4b: Antibacterial activity of Cobalt (II) complexes of pyrazine-2-carboxylic acid hydrazide and its various derivatives against gram positive and gram negative organisms

S. No.	Compound	Gram Positive Organisms			Gram Negative Organism	
		S.aureus	B.subtilis	B.pumilus	E.coli	K.pneumoniae
		Diameter of Zone of Inhibition (mm)	Diameter of Zone of Inhibition (mm)	Diameter of Zone of Inhibition (mm)	Diameter of Zone of Inhibition (mm)	Diameter of Zone of Inhibition (mm)
1	$[Co(C_5H_6N_4O)_2Cl_2]$	14	7.8	7.8	12	12.4
2	$[Co(C_{12}H_{10}N_4O)_2Cl_2]$	11.2	7.8	12.2	14.1	15.1
3	$[Co(C_{13}H_{12}N_4O_2)_2Cl_2]$	14.1	7.8	14.1	15.7	17.8
4	$[Co(C_{13}H_{12}N_4O_3)_2Cl_2]$	15.3	7.8	13.3	13.3	17
5	$[Co(C_{16}H_{19}N_5O)_2Cl_2]$	14.1	7.8	11.5	14.4	7.8
6	$[Co(C_{14}H_{12}N_4O)_2Cl_2]$	13	7.8	7.8	13	7.8
7	$[Co(C_{13}H_{11}N_4O_2)_2]$	7.8	7.8	17	13.3	12.1

(1) Zones of inhibition (mm) are the mean of three experiment observations.

Table 4c: Antibacterial activity of Nickel (II) complexes of pyrazine-2-carboxylic acid hydrazide and its various derivatives against gram positive and gram negative organisms

S. No.	Compound	Gram Positive Organisms			Gram Negative Organism	
		S.aureus	B.subtilis	B.pumilus	E.coli	K.pneumoniae
		Diameter of Zone of Inhibition (mm)	Diameter of Zone of Inhibition (mm)	Diameter of Zone of Inhibition (mm)	Diameter of Zone of Inhibition (mm)	Diameter of Zone of Inhibition (mm)
1	$[Ni(C_5H_6N_4O)_2Cl_2]$	14	7.8	14	12	20.2
2	$[Ni(C_{12}H_{10}N_4O)_2Cl_2]$	14	7.8	7.8	14.2	14.2
3	$[Ni(C_{13}H_{12}N_4O_2)_2Cl_2]$	16.4	7.8	7.8	12.1	7.8
4	$[Ni(C_{13}H_{12}N_4O_3)_2Cl_2]$	14.1	7.8	15.3	12	7.8
5	$[Ni(C_{16}H_{19}N_5O)_2Cl_2]$	14.3	7.8	7.8	12.4	7.8

(1) Zones of inhibition (mm) are the mean of three experiment observations.

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Evaluation of antibacterial activity: All the manganese (II), cobalt (II) and nickel (II) complexes of pyrazine-2-carboxylic acid hydrazide and its hydrazones (PAH derivatives) were screened for antibacterial activity against gram positive (S. aureus, B. subtilis and B. pumilus) and gram negative (E. coli and K. pneumoniae) organisms.

The activities are represented by mean of diameter (mm) of zones of inhibition, based on average of three experimental observations.

From data it is clear that all the chelates are inactive against B. subtilis and general trend of activity is E. coli > S. aureus > K. pneumonia > B. pumilus and majority of chelates of Mn (II), Co(II) and Ni(II) complex do not show activity against B. pumilus and K. pneumonia. In general, activity is decreased on chelation.

4. Conclusion

In view of the facts that ligands possess antibacterial properties, the present studies were carried out to study the antibacterial behavior of metal complexes. After formation of complexes the effect as antibacterial may increase or decrease. However, in present case the activity as antibacterial has decreased after formation of complex. This is probably due to lesser activity as antibacterial of individual metal in comparison to the free ligands.

5. Acknowledgements

The author feels indebted and grateful for the technical help and valuable suggestions given by Dr. R. K. Bhardwaj, Associate Professor of Chemistry, D. S. College Karnal (Haryana) India, Dr. S. N. Mittal, Associate Professor of Chemistry, M. L.N. College Yamuna Nagar (Haryana) India and Dr. P. K. Sehgal, Zoologist, Dehradun (Uttara Khand) India in preparing and presenting this paper.

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