

SYNTHESIS, CHARACTERIZATION AND BIOCHEMICAL STUDIES OF SOME COPPER (II) AND NICKEL (II) COMPLEXES WITH A SCHIFF BASE LIGAND

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ABSTRACT: A new series of complexes was synthesized by template condensation of propane dihydrazide and 2,3-Butanedione in methanolic medium in the presence of divalent copper and nickel salts, whereby complexes of the type : $[M(H_2L)X_2]$ Where $M = Cu(II)$ and $Ni(II)$, $L = 1,2,5,6$ -teraza-1,6-dihydro-3,4-dimethyl-2,4-diene-7,9-cyclononadiene and $X = Cl^-$, NO_3^- and CH_3COO^- , were formed. The complexes were characterized with the aid of elemental analyses, conductance measurements, magnetic susceptibility measurements, and electronic and infrared spectral studies. Based on these studies, a six coordinate octahedral geometry is proposed for these complexes. The complexes were tested for their *in vitro* antibacterial and antifungal activities. The minimum inhibitory concentration shown by complexes was compared with that of standard drugs.

Keywords: Antibacterial; Antifungal; Macrocyclic complexes; Minimum inhibitory concentration.

INTRODUCTION

To overcome the alarming problem of microbial resistance to antibiotics, the discovery of novel active compounds against new targets is a matter of urgency. Many of the crude drugs, which are sources of medicinal preparations, still originate from wild-growing material. However, macrocyclic have shortened the life span of the source of material. Macrocyclic complexes are of great interest due to their resemblance to naturally occurring macrocycles and analytical, industrial and medical applications.¹⁻⁴

In the present work, complexes of $Cu(II)$ and $Ni(II)$ with a Schiff base ligand have been synthesized. Their structures were confirmed by IR spectral analysis. Further, their antibacterial activity towards some clinically important bacteria was evaluated⁵⁻⁷.

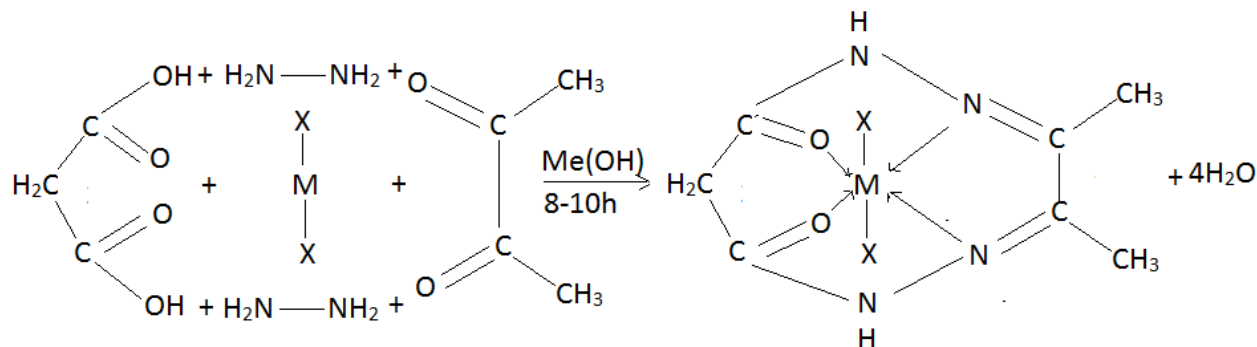
EXPERIMENTAL

Isolation of the complexes

The complexes were synthesized by the template method, *i.e.*, by condensation of propanedihydrazide and 2,3-Butanedione in the presence of a divalent metal salt. To a hot stirred methanolic solution (≈ 50 mL) of propanedihydrazide (10 mmol) was added a divalent copper and nickel salt (Cl^- , NO_3^- , CH_3COO^- (10mmol) dissolved in the minimum quantity of

methanol (≈ 20 mL). The resulting solution was boiled under reflux for 0.5 h. Subsequently, 2,3-Butanedione (10 mmol) was added to the refluxing mixture and refluxing was continued for 8–10 h. The mixture was concentrated to half its volume and kept in a desiccators overnight. The complexes were then filtered, washed with methanol, acetone and ether and dried **in vacuo**; yield ≈ 50 – 60 %. The complexes were soluble in DMF and DMSO, but insoluble in other common organic solvents and water. They were found to be thermally stable up to ≈ 225 – 260 °C, after which they decomposed.

The template condensation of propanedihydrazide and 2,3-Butanedione in the presence of divalent copper and nickel salts may be represented by the following scheme:



Where M=

Cu (II) and Ni (II), and X= Cl⁻, NO₃⁻, CH₃COO⁻,

Analytical and physical measurements

The microanalyses for C, H, and N were realized at SAIF, CDRI, Lucknow. The metal contents were determined by standard EDTA methods. The electronic spectra (DMF) were recorded on a Cary 14 spectrophotometer. The magnetic susceptibility measurements were performed by Guoy method. The IR spectra were recorded on an FT-IR spectrophotometer (Perkin Elmer) in the range 4000–200 cm⁻¹ using the Nujol Mull method, at B.R. Ambedkar Bihar University, Muzaffarpur. The conductivity was measured on a digital conductivity meter (HPG System, G-3001).

In-vitro antibacterial activity

Primary screening. The antibacterial activities of the newly synthesized complexes were evaluated by the Agar Well Diffusion Assay Technique against two Gram-positive bacteria, *i.e.*, *Bacillus subtilis* (MTCC 8509) and *Bacillus stearothermophilus* (MTCC 8508) and two Gram-negative bacteria, *i.e.*, *Escherichia coli* (MTCC 51) and *Pseudomonas putida* (MTCC121). The bacterial cultures were maintained on the nutrient agar media by sub-culturing them on fresh slants after every 4–6 weeks and incubating them at the appropriate temperature for 24 h. All stock cultures were stored at 4 °C. For the evaluation of antimicrobial activity of the synthesized complexes, a suspension of each test microorganism was prepared. The turbidity of each suspension was adjusted to 0.5 McFarland units by suspending the cultures in sterile distilled water. The size of final inoculum was adjusted to 5×10^7 CFU ml⁻¹. A volume of 20 ml of agar media was poured into each Petri plate and the plates were swabbed with broth cultures of the respective micro-organisms and kept for 15 min for adsorption to occur. Using a punch, ≈ 8 mm diameter wells were bored in the seeded agar plates and a 100 μ l volume of each test compound reconstituted in DMSO was added into the wells. DMSO was used as the control for all the test complexes. After holding the plates at room temperature for 2 h to allow diffusion of the

compounds into the agar, the plates were incubated at 37 °C for 24 h. Antibacterial activity was determined by measuring the diameter of the inhibition zone. The entire tests were performed in triplicate and the mean of the diameter of inhibition was calculated. The antimicrobial activities of the complexes were compared against standard drugs.

Minimum Inhibitory Concentration (MIC).

Nutrient broth was adjusted to pH 7.0 for the determination of the MIC of synthesized complexes. The MIC is the lowest concentration of an antimicrobial agent that prevents the development of visible growth of microorganism after overnight incubation. The inoculum of the test microorganisms were prepared using 16 h-old cultures adjusted by reference to the 0.5 McFarland standards (10^8 cells ml⁻¹).⁹ These cultures were further diluted up to 10-fold with nutrient broth to obtain an inoculum size of 1.2×10^7 CFU ml⁻¹. A positive control (containing inoculum but no complex) and a negative control (containing complex but no inoculum) were also prepared. A stock solution of 4 mg ml⁻¹ of each compound was prepared in DMSO and further appropriately diluted to obtain final concentrations ranging from 250 to 0.03 µg ml⁻¹.⁷ The requisite quantity of antifungal drug (cyclohexamide) was added to the broth to obtain its desirable final concentration of 100 µg ml⁻¹. Separate flasks were taken for each test dilution. To each flask was added 100 µl of inoculum. Then the appropriately diluted test sample was added to each flask having broth and microbial inoculum. The contents of the flask were mixed and incubated for 24 to 48 h at 37 °C. The test bacterial cultures were spotted in a predefined pattern by aseptically transferring 5 µl of each bacterial culture onto the surface of solidified agar-agar plates and incubated at 37 °C for 24 h for determining the MIC value.

IN-VITRO ANTIFUNGAL ACTIVITY:

Potato dextrose medium (PDA) was prepared in a flask and sterilized. To check the growth of bacterial culture in the medium, the requisite quantity of the standard antibiotic (ampicillin) was added to obtain the desired final concentration of 100 µg ml⁻¹ of the medium. Test samples were prepared in different concentrations (10, 50 and 100 µg/ml) in dimethyl sulphoxide and 200 µl of each sample was spread on the PDA media contained in sterilized Petri plates. Mycelial discs taken from the standard cultures of fungi (*Aspergillus flavus* and *A. niger*) were grown on the PDA medium for 5–7 days. These cultures were used for the aseptic inoculation in the sterilized Petri dish. Standard cultures inoculated at 28±1 °C were also used as the control. The efficacy of each sample was determined by measuring the radial mycelial growth. The radial growth of the colony was measured in two directions at right angle to each other and the average of two replicates was recorded in each case. The data are expressed as percent inhibition over the control obtained from the size of colonies. The percent inhibition was calculated using the formula:

$$\% \text{ Inhibition} = 100(C-T)/C$$

where C is the diameter of fungus colony in the control plate after incubation for 96 h and T is the diameter of the fungus colony in the tested plate after the same incubation period.

RESULTS AND DISCUSSION

The analytical data show the formula for macrocyclic complexes as:



Where $M = \text{Cu(II) Ni(II)}$,

$X = \text{Cl}^-, \text{NO}_3^-, \text{or CH}_3\text{COO}^-$ and

$L = 1,2,5,6\text{-teraaza-}1,6\text{-dihydro-}3,4\text{-dimethyle}2,4\text{-diene-}7,9\text{-cyclononadiene}$ (AHMEDO)

The tests for the anions were positive only after decomposition of the complexes, indicating their presence inside the coordination sphere. All macrocyclic complexes were dark-coloured solids, which were soluble in DMF and DMSO. The measurements of molar conductance (conductance $\approx 10\text{--}20 \text{ S cm}^2 \text{ mol}^{-1}$) in DMSO showed that these chelates are non-electrolytes.⁸

All complexes gave satisfactory elemental analysis as shown in Table (I).

Table (I)

Analytical data of the complexes

Complexes	Colour	Found(Calc.)				
		Metal	C	H	N	Cl ⁻
Ni(H ₂ L)Cl ₂	Brown	18.63 (18.74)	26.74 (26.87)	3.15 (3.20)	17.82 (17.91)	22.61 (22.71)
Ni(H ₂ L)(NO ₃) ₂	Light brown	15.87 (16.07)	22.96 (23.03)	2.66 (2.74)	22.95 (23.03)	-
Ni(H ₂ L)(CH ₃ COO) ₂	Shine black	16.25 (16.34)	36.62 (36.80)	4.30 (4.46)	15.51 (15.61)	-
Cu(H ₂ L)Cl ₂	Light brown	19.98 (20.07)	26.48 (26.53)	3.10 (3.15)	17.53 (17.69)	22.30 (22.43)
Cu(H ₂ L)(NO ₃) ₂	Light brown	17.10 (17.19)	22.50 (22.72)	2.68 (2.70)	22.60 (23.73)	-
Cu(H ₂ L)(CH ₃ COO) ₂	Shine black	17.36 (17.47)	36.20 (36.30)	4.30 (4.40)	15.35 (15.40)	-

IR SPECTRA : In the IR spectrum of propane dihydrazide, a pair of bands corresponding to $\nu(\text{NH}_2)$ was present at ≈ 3210 and $\approx 3270 \text{ cm}^{-1}$, but absent in the IR spectra of all the complexes. However, a single broad medium band at $3360\text{--}3450 \text{ cm}^{-1}$ was observed in the spectra of all the complexes, which may be assigned to $\nu(\text{NH})$ stretching vibrations. It suggested that NH group does not take part in coordination. A strong peak at $\approx 1660 \text{ cm}^{-1}$ in the IR spectrum of propanedihydrazide is assigned to the $>\text{C}=\text{O}$ group of the CONH moiety. This peak is shifted to lower frequencies ($\approx 1620\text{--}1640 \text{ cm}^{-1}$) in the spectra of all the complexes, suggesting the coordination of the oxygen of the carbonyl group with the metal. Furthermore, no strong

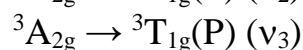
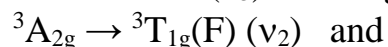
absorption band was observed near 1700 cm^{-1} in the IR spectra of the complexes but was observed in the spectrum of 2,3-Butanedione.

This indicates the absence of $>\text{C}=\text{O}$ groups of the 2,3-Butanedione moiety in the complexes. These facts confirm the condensation of carbonyl groups of 2,3-Butanedione and the amino groups of propane dihydrazide. The IR spectra of the complexes showed a new strong absorption band in the region $\approx 1595\text{--}1610\text{ cm}^{-1}$, which may be attributed to the $\nu_{(\text{C}=\text{N})}$ group. These results provide strong evidence for the formation of the macrocyclic frame. The lower value of $\nu_{(\text{C}=\text{N})}$ indicates coordination of the nitrogen of azomethine to the metal. The bands present at $\approx 1300\text{--}1000\text{ cm}^{-1}$ are assigned to $\nu(\text{C}-\text{N})$ vibration. The bands presents at $\approx 3040\text{ cm}^{-1}$ may be assigned to $\nu(\text{C}-\text{H})$ vibrations of the 2,3-Butanedione moiety. The IR spectra of the nitrate complexes display three (N-O) stretching bands at $\approx 1410\text{--}1455\text{ cm}^{-1}$ (ν_5), $\approx 1305\text{--}1315\text{ cm}^{-1}$ (ν_1) and $\approx 1015\text{--}1030\text{ cm}^{-1}$ (ν_2). The separation of the two highest frequency bands ($\nu_5 - \nu_1$) suggest that both the nitrate groups are coordinated in a unidentate manner.⁹ The acetate complexes showed two bands at $\approx 1630\text{--}1640\text{ cm}^{-1}$ (ν_1) and $\approx 1380\text{--}1390\text{ cm}^{-1}$ (ν_2). These indicate that the acetate group is coordinated in a unidentate manner.

The far IR spectra show bands in the region $\approx 420\text{--}450\text{ cm}^{-1}$, corresponding to $\nu(\text{M}-\text{N})$ vibrations in all the complexes.¹⁰ The presence of a band in all the complexes in the $\approx 420\text{--}450\text{ cm}^{-1}$ region originate from (M-N) azomethine vibration modes and support the coordination of azomethine nitrogen with the metal. The bands present at $\approx 310\text{--}315\text{ cm}^{-1}$ in the chloride complexes are due to $\nu(\text{M}-\text{Cl})$, and bands present at $\approx 210\text{--}240\text{ cm}^{-1}$ in all the nitrate complexes are due to $\nu(\text{M}-\text{O})$.

MAGNETIC MEASUREMENTS AND ELECTRONIC SPECTRA:

Nickel complexes. The magnetic moment of the nickel complexes at room temperature lie in the range $2.91\text{--}2.95\ \mu_{\text{B}}$ showing an octahedral environment around the Ni(II) ion in all complexes. The solution spectra of the Ni(II) complexes exhibited a well-discernible band with a shoulder on the low energy side. The other two bands generally observed in the region at *ca.* $16570\text{--}17240\text{ cm}^{-1}$ (ν_2) and $26860\text{--}28000\text{ cm}^{-1}$ (ν_3) are assigned to



transitions, respectively. The first two bands result from the splitting of one band, ν_1 , are in the range $\approx 9700\text{--}10000$ and $11800\text{--}12440\text{ cm}^{-1}$, which can be assigned to ${}^3\text{B}_{1g} \rightarrow {}^3\text{E}_g$ and ${}^3\text{B}_{1g} \rightarrow {}^3\text{B}_{2g}$ transitions, respectively, assuming the effective symmetry to be D_{4h} (component of ${}^3\text{T}_{2g}$ in O_h symmetry). The intense higher energy band at *ca.* 34000 cm^{-1} may be due to a $\pi\text{-}\pi^*$ transition of the (C=N) group. The various bands do not follow any regular pattern and seem to be anion independent. The spectra are consistent with the distorted octahedral nature of these complexes. Various ligand field parameters, Dq , B , β and $\beta\%$ were calculated for the complexes. B for a free nickel(II) ion is 1040 cm^{-1} . The values of β lie in the range $0.509\text{--}0.519$. These

values indicate the presence of covalent character in the metal–ligand “ σ ” bond. The value of the v_2/v_1 ratio lies between 1.37–1.41 and shows that the complexes possess a distorted octahedral structure.

Copper complexes: The magnetic moment of the copper complexes lie in the range 1.77–1.82 μB . The electronic spectra of the copper complexes exhibit bands in the region *ca.* 17780–19000 cm^{-1} with a shoulder on the low energy side at \approx 14600–16000 cm^{-1} , which show that these complexes have a distorted octahedral geometry.¹¹⁻¹³ Assuming tetragonal distortion in the molecule, the d-orbital energy level sequence for these complexes may be: $x^2 - y^2 > z^2 > xy > xz > yz$ and the shoulder can be assigned to: $z^2 \rightarrow x^2 - y^2$ (${}^2\text{B}_{1g} \rightarrow {}^2\text{B}_{2g}$) and the broad band contains both $xy \rightarrow x^2 - y^2$ (${}^2\text{B}_{1g} \rightarrow {}^2\text{E}_g$) and $xz, yz \rightarrow x^2 - y^2$ (${}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{2g}$) transitions. The band separation of the spectra of the complexes is of the order 2500 cm^{-1} , which is consistent with the proposed geometry of the complexes. Therefore, it may be concluded that all the complexes formed by the macrocycles with Cu(II) metals are distorted octahedral.

Biological results and discussion:

In this study, all the chemically synthesized complexes were evaluated against Gram-positive and Gram-negative bacteria. The *MIC* values of the synthetic complexes were determined by the method given by Andrews¹⁴. Standard antibiotics, namely streptomycin and chloramphenicol, were used for comparison with the antibacterial activities exhibited by these complexes. All the complexes of the tested series possessed some antibacterial activity against Gram-positive bacteria as well as Gram-negative bacteria. In the whole series, complexes **1** and **5** were found to be most effective against all the tested bacterial strains, showing zone of growth inhibition in the range from 46.2–49.2 mm. *In-vitro* antibacterial activity of the complexes determined by the agar well diffusion method for a concentration of 100 $\mu\text{g ml}^{-1}$ (A – *Bacillus subtilis* (MTCC 8509), B – *Bacillus stearothermophilus* (MTCC 8508), C – *Escherichia coli* (MTCC 51), D – *Pseudomonas putida* (MTCC 121)) and 36.2–38.2 mm, respectively. Complexes **9** and **11** exhibited good activity against all the tested bacterial strains with a zone of inhibition in the range from 21.2–27.3 mm and 21.4–28.8 mm, respectively. Complex **2** showed the highest zone of inhibition (29.6 and 28.1 mm) against *E. coli* and *P. putida*, respectively. Complex **7** showed the highest zone of inhibition 23.2–26.4 mm against *B. subtilis*, *E. coli* and *P. putida*. Based on the *MIC* values shown by these complexes against all the bacterial strains, complex **1** was found to be most effective by showing a *MIC* of 4 $\mu\text{g ml}^{-1}$ for *E. coli*, which is equal to the *MIC* shown by the standard antibiotic chloramphenicol and streptomycin against the same bacterial strain. Complex **1** also exhibited *MIC* values in the range from 4 to 8 $\mu\text{g ml}^{-1}$ for the other bacterial strains.

In the whole series, the *MIC* value of complex **5** was found to be 16 $\mu\text{g ml}^{-1}$ for *E. coli* and 32 $\mu\text{g ml}^{-1}$ for *B. subtilis*, *B. stearothermophilus* and *P. putida*. Complex **11** showed an *MIC* of 64 $\mu\text{g ml}^{-1}$ for *B. subtilis*. Minimum inhibitory concentration (*MIC*) shown by the complexes against the test bacteria determined by agar dilution assay (A – *Bacillus subtilis* (MTCC 8509), B – *Bacillus stearothermophilus* (MTCC 8508), C – *Escherichia coli* (MTCC 51), D – *Pseudomonas putida* (MTCC 121))

The antifungal activities of all the complexes were determined against two fungal strains, *i.e.*, *A. flavus* and *A. niger*, and then compared with the standard antifungal drug cyclohexamide. In the whole series, complex **1** showed the highest percentage inhibition (41–43 %) against both fungal strains but none of the tested complex restricted fungal growth excellently. However, of all the tested complexes, complex **5** showed nearly 30–31 % inhibition of mycelial growth against both fungal strains *i.e.*, *A. flavus* and *A. niger*, whereas complexes **10** and **11** showed nearly 15–24 % inhibition of mycelial growth against *A. flavus* and *A. niger*.

No.	Complexes	Diameter of zone of growth inhibition mm			
		A	B	C	D
1.	Ni(H ₂ L)Cl ₂	10.43	19.32	16.56	20.86
2.	Ni(H ₂ L)(NO ₃) ₂	36.03	37.11	38.26	36.29
3.	Ni(H ₂ L)(CH ₃ COO ⁻) ₂	9.66	12.30	20.93	19.65
4.	Cu(H ₂ L)Cl ₂	13.00	24.05	25.91	23.30
5.	Cu(H ₂ L)(NO ₃) ₂	14.40	13.04	14.20	21.45
6.	Cu(H ₂ L)(CH ₃ COO ⁻) ₂	22.02	26.91	23.08	21.06

CONCLUSIONS Based on elemental analyses, conductivity and magnetic measurements, and electronic, IR spectral studies, the structure shown in Fig. 1

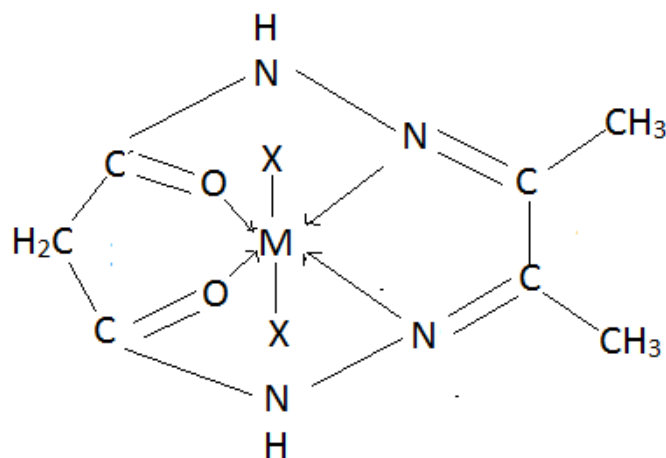


Fig-1

may be proposed for all the synthesized complexes. However, none of the synthesized macrocyclic metal complexes showed good antibacterial and antifungal activities against all the bacterial and fungal strains, but some of the complexes, were found to be most effective against various bacterial and fungal strains. It is suggested that chelation/ coordination reduces the polarity of the metal ion, mainly because of the partial sharing of its positive charge with a donor group within the whole chelate ring system. This process of chelation thus increases the lipophilic nature of the central metal atom, which in turn, favours its permeation through the lipid layer of the membrane, thus causing the metal complex to cross the bacterial membrane more effectively, thus increasing the activity of the complexes. In addition to this, many other factors, such as solubility, dipole moment and conductivity, which are influenced by the metal ion may be the possible reasons for the antibacterial activities of these metal complexes. It was also observed that some moieties, such as azomethine linkage or heteroaromatic nucleus introduced into such compounds exhibit extensive biological activities that may be responsible for the increase in hydrophobic character and liposolubility of the molecules in crossing the cell

membrane of the microorganism and enhance the biological utilization ratio and activity of the complexes¹⁴.

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