# SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF SOME MIXED LIGAND Co(II) and Ni(II) COMPLEXES

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**Abstract:** Mixed ligand Co(II) and Ni(I) complexes have been synthesized by using 8–hydroxyquinoline as primary ligand and N– and/or O– donor ligands such as tartaric acid/phenylalanine as secondary ligands. The metal complexes have been characterized on the basis of elemental analysis, electrical conductance, room temperature magnetic susceptibility measurements, spectral and thermal studies. The electrical conductance studies of the complexes in methanol solution at $10^{-3}$ M concentration indicate their non–electrolytic nature. Room temperature magnetic susceptibility measurements are indicative of an octahedral geometry for Co(II) and Ni(II) complexes. Electronic absorption/reflectance spectra of the complexes show intra–ligand, charge transfer and the crystal field transitions, which are supportive of the proposed octahedral geometries of the complexes. The thermal analysis data of the complexes indicate the presence of crystallization water. The antibacterial and antifungal activities of the complexes have been studied against some of the pathogenic bacteria and fungi. The cup–plate method has been used to study the antibacterial activity of the compounds against *C. diphtheriae, E. coli, S. typhi, S. dysentariae, S. aureus* and *V. cholerae*. The results have been compared against those of controls, which were screened simultaneously. The activity is measured by measuring the diameter of the inhibited zone in millimeters (mm). The antifungal activity of the complexes against *Candida albicans* and *Aspergillus niger* has been studied by the tube dilution method. The complexes have been screened for acute oral toxicity in rats. The $LD_{50}$ values have been determined by the method of Litchfield and Wilcoxon.

**Keywords:** cobalt, nickel, mixed ligand complexes, antimicrobial activity, $LD_{50}$

The use of 8–hydroxyquinoline as an *in vivo* agent in microbiological system has been reviewed by Schulman and Dwyer (1). The majority of the metal complexes possessing biological activity are chelates (2). Several reviews on the relationship of metal complexes to biological response have been reported (3). The antimicrobial activity of some mixed ligand Co(II) complexes against some pathogenic microorganisms has also been reported (4).

The present paper reports the synthesis and characterization of mixed ligand Co(II)/Ni(II) complexes prepared by using 8–hydroxyquinoline as primary ligand and some N– and/or O–donor ligands as secondary ligands. The complexes have been screened for their antibacterial and antifungal activity and the results have been compared with those of the controls. Acute oral toxicity studies on these complexes have been carried out in rats.

## EXPERIMENTAL

### Materials

Most of the chemicals used were of AR grade. Laboratory grade chemicals, whenever used, were purified by standard methods, while the solvents were purified and double distilled before use.

### Preparation of mixed ligand complexes

The mixed ligand Co(II)/Ni(II) complexes were prepared from cobalt(II) chloride/nickel(II) chloride, 8–hydroxyquinoline (HQ) and chiral secondary ligands such as (+)tartaric acid/(–)phenylalanine.

To a blue coloured ethanolic (10 ml) solution of cobalt(II) chloride hexahydrate (237 mg, 1 mmole), an ethanolic (10 ml) solution of 8–hydroxyquinoline (145 mg, 1 mmole) was added. The mixture was stirred and kept in a boiling water bath for 10 minutes, during which time it turned green in colour. To this mixture, an aqueous (10 ml) solution of tartaric acid (150 mg, 1 mmole) was added. The mixture (1:1:1 molar proportion) was heated in a hot water bath. The complexes were obtained by raising the pH of the reaction mixture by adding dilute NaOH solution. The mixture was cooled and the solid was filtered, washed with ice–cold water followed by aqueous ethanol. The complexes thus prepared were dried under vacuum. The mixed

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ligand Ni(II) complex was prepared from nickel (II) chloride, 8–hydroxyquinoline (HQ) and phenylalanine, by the same method described above.

## Instrumentation

The complexes were analysed for the metal contents, C, H and N using standard procedures. The molar conductance values were measured in methanol ($10^{-3}$ M) on a model CM–180 Elico digital conductivity meter. Room temperature magnetic susceptibilities were measured by a Guoy balance using $Hg[Co(SCN)_4]$ as the calibrant. Effective magnetic moments were calculated after applying diamagnetic corrections for the ligand components using Pascal's constants (5). Electronic absorption spectra in methanol and chloroform solution in the ultraviolet and visible range, respectively, were measured on a Shimadzu UV–160A and Spectronic–20 spectrophotometer. Reflectance spectra of the solid complexes in the visible region were recorded against $BaSO_4$ on a Shimadzu UV–2100 spectrophotometer. FT–IR spectra were recorded in KBr discs on a Perkin–Elmer FT–IR spectrophotometer model 160. Thermal studies of the complexes were made on a Mettler TC 10A TA processor by recording the change in weight of the complexes on the increasing temperature up to 700°C at a heating rate of 10°C/min.

## Antimicrobial screening

The minimum inhibition concentrations (MIC) of the two complexes were determined by using the cup–plate method described elsewhere (6). Tube dilution method, described elsewhere (7), was used to study antifungal activity.

## Acute toxicity studies

All the test samples were prepared as suspensions by using distilled water as solvent.

Acute oral toxicity study of these samples was carried out in albino rats (80–120 g) of either sex maintained under standard husbandry conditions. Ten rats per group were used for all sets of experiments. The animals were allowed to take standard laboratory feed and water *ad libitum*. The animals were divided into control and test groups. The test groups received various test substances orally in different doses and were observed for mortality till 24 hours. To all control animals 0.5 ml of vehicle (sterile water) was given as a control. The $LD_{50}$ values were determined by the method of Litchfield and Wilcoxon (8).

## RESULTS AND DISCUSSION

### Characterization of metal complexes

The synthesis of the mixed ligand Co(II)/Ni(II) complexes may be represented as follows:

$$Co(II)/Ni(II) + HQ + HL \longrightarrow Co(II)/Ni(II)QL$$
$$1 \quad : \quad 1 \quad : \quad 1 \qquad \text{mixed ligand}$$
$$\text{complex}$$
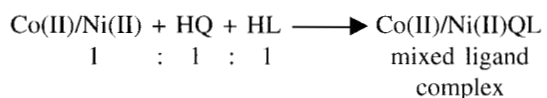
(where HQ is 8–hydroxyquinoline and HL is a chiral secondary ligand)

Both the complexes are non–hydroscopic stable solids. They are coloured and thermally stable (Table 1), indicating a strong metal–ligand bond. The elemental analysis data (Table 1) of metal complexes is consistent with their general formulation as mixed ligand complexes $MQL \cdot H_2O$, where M is the divalent cobalt/nickel ion, Q is the deprotonated 8–hydroxyquinoline and L is the deprotonated optically active compound. The molar conductance values of the complexes in methanol at $10^{-3}$ M concentration are very low (<1), indicating their non–electrolytic nature (9).

Table 1. Colour, decomposition temperature, magnetic moment, molar conductance and analytical data of the metal complexes[a].

| Compound | Empirical formula | Colour | Decomp. temp. (°C) | Elemental analysis Found (Calculated) | | | | Molar Cond. Ohm×cm² ×mol⁻¹ | $\mu_{eff}$ (B.M.) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | %M | %C | %N | %H | | |
| $[Co(Q)(Tar)] \cdot H_2O$ | $CoC_{13}H_{13}O_8N$ | Light yellow | >300 | 15.72 (15.93) | 42.35 (42.17) | 3.75 (3.78) | 3.71 (3.51) | 0.43 | 4.92 |
| $[Ni(Q)(Phe)] \cdot H_2O$ | $NiC_{18}H_{18}O_4N_2$ | Green | 200 | 15.41 (15.25) | 56.01 (56.15) | 7.25 (7.27) | 4.50 (4.68) | 0.52 | 2.82 |

[a]Q represents the deprotonated primary ligand 8–hydroxyquinoline whereas Tar and Phe represent the deprotonated secondary ligands tartaric acid and phenylalanine, respectively.

Table 2. Some important infrared spectral bands (cm⁻¹) of Co(II)/Ni(II) complexes[a].

| Compound | $\nu$(O–H) (H₂O) | $\nu_a$(N–H) (A. a.) | $\nu_s$(N–H) (A. a.) | $\nu$(C=O) | $\nu$(C–O) | $\nu$(HOH) | $\nu$(C=N) (HQ) | $\nu$(C–O) (HQ) | $\nu$(C–N) (A. a.) | $\nu$(M–N) | $\nu$(M–O) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| [Co(Q)(Tar)]·H₂O | 3303 w | – | – | 1636 m | 1106 s | 1576 m | 1499 m | 1106 s | – | 605 w | 415 w |
| [Ni(Q)(Phe)]·H₂O | 3320 w | 3209 w | 3075 w | 1639 s | 1379 s | 1577 s | 1499 s | 1107 s | 907 w | 602 w | 405 w |

[a] s: strong, m: medium, w: weak.

## Infra–red spectra

The FT–IR spectra of the CML metal complexes, recorded in KBr in the of 4000–400 cm⁻¹ are quite complex due to the presence of numerous bands of varying intensities, rendering the task of their assessment quite difficult. However, an attempt has been made to assign some of the important bands on the basis of reported infra–red spectra of tartaric acid, phenylalanine, 8–hydroxyquinoline and their metal complexes (4,10–12).

1. A broad band observed in the region between 3320–3303 cm⁻¹ due to antisymmetric and symmetric O–H stretching modes and a strong peak in the range 1577–1576 cm⁻¹ due to H–O–H bending showing the presence of crystallization water.

2. The protonated non coordinated carboxyl group in tartaric acid has an absorption at 1740 cm⁻¹, which is typical of C=O stretching vibrations of –COOH. The corresponding coordinated carboxylate in the complex shows absorption at lower frequency, i.e. 1636 cm⁻¹, which leads to conclusion that the complexation takes place by deprotonation of carboxylic group of tartaric acid moiety. The shifting of the stretching and bending frequency $\nu$(C–O) towards lower wave number, i.e. 1106 cm⁻¹ in Co(Q)(Tar), indicates the coordination of secondary –OH group via oxygen with metal.

3. Broad bands at 3209 and 3075 cm⁻¹ are respectively due to N–H (asym.) and N–H (sym.) vibrations of phenylalanine moiety. The antisymmetric stretching frequencies $\nu$(C=O) and $\nu$(C–O) of phenylalanine are shifted to lower wave numbers, i.e. 1639 and 1379 cm⁻¹ respectively, in metal complexes indicating the coordination of carboxylic acid group via oxygen to the metal. The shift in the frequency of carbon–nitrogen bond at ~907 cm⁻¹ (symmetric stretching) is indicative of coordination through the amino group of amino acid.

4. Charles et al. (13) reported that for several metal complexes with HQ, $\nu$(C–O) band is observed at ~1120 cm⁻¹. The position of this band undergoes variation depending on the metal complex under study. A strong $\nu$(C–O) band is observed in the range 1107–1106 cm⁻¹ indicating the presence of oxine moiety in the complexes coordinating through its nitrogen and oxygen atoms as uninegative bidentate ligand. The $\nu$(C=N) mode in oxine occurs at 1499 cm⁻¹ in the spectra of metal complexes. This band is observed in the spectrum of the ligand in the higher region (1580 cm⁻¹). A negative shift in this vibrational mode on complexation indicates the coordination through the tertiary nitrogen donor of HQ. The *in plane* and *out of plane* ring deformation modes are observed at ~505 and ~787 cm⁻¹ respectively, confirming coordination through the nitrogen atom of HQ with metal.

5. The M–O bond has much less covalent character than the M–N bond so the stretching bands of the former bond appear in low frequency region. The new bands of weak to medium intensity observed in the regions around 602–605 cm⁻¹ and 405–415 cm⁻¹ may be ascribed to M–N and M–O vibrations, respectively. It may be noted that these vibrational bands are absent in the infra–red spectra of primary and secondary ligands.

Some of the important IR bands and their tentative assignments are shown in Table 2.

## Magnetic susceptibility measurements

The magnetic moments of the mixed ligand metal complexes, calculated from the measured magnetic susceptibilities after employing diamagnetic corrections, are given in Table 1. The observed $\mu_{eff}$ values for the Co(II) complex with tartaric acid and Ni(II) complex with phenylalanine are 4.92 and 2.82 B.M., respectively, which are suggestive of an octahedral geometry (14,15) for these complexes. The magnetic moments of the compounds investigated are in agreement with the conclusions based on spectral findings, discussed later.

Table 3. Diffuse reflectance spectral data (cm⁻¹) for mixed ligand Co(II)/Ni(II) complexes.

| Compound | $v_1$* | $v_2$ | $v_3$ | DQ | B' | β |
|---|---|---|---|---|---|---|
| [Co(Q)(Tar)]·H₂O | 7.838 | 16.806 | 20.000 | 896.7 | 885.9 | 0.912 |
| [Ni(Q)(Phe)]·H₂O | 6.901 | 12.706 | 18.939 | 690.1 | 729.4 | 0.700 |

*calculated value.

Table 4. Thermal data for metal complexes.

| Compound | Temperature range (°C) | Weight loss due to H₂O (%) | |
|---|---|---|---|
| | | Calculated | Observed |
| [Co(Q)(Tar)]·H₂O | 111–181 | 4.9 | 5.0 |
| [Ni(Q)(Phe)]·H₂O | 119–170 | 4.7 | 4.8 |

## Electronic absorption spectra

The electronic spectra of the two metal complexes in methanol solution, recorded in the ultraviolet region, exhibit intra–ligand and charge transfer transitions in the range around 29.673–48.192 cm⁻¹ and near 26.000 cm⁻¹, respectively. The electronic absorption spectrum of Co(II) complex in chloroform solution in the visible and near infra red region shows three transition bands in the region around 25.000 cm⁻¹, 16.666 cm⁻¹ and 12.000 cm⁻¹, respectively, while that of the Ni(II) complex shows two transition bands in the region around 25.000 cm⁻¹ and 12.195 cm⁻¹, respectively.

The diffuse reflectance spectrum of the Co(II) complex with tartaric acid shows the d–d transition bands in the region around 16.806 and 20.000 cm⁻¹, which are assigned to transitions $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ (v2) and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ (v3), respectively. The Ni(II) complex shows two transition bands in the region around 12.706 and 18.939 cm⁻¹, which are ascribed to transitions $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ (v2) and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ (v3), respectively. As the $v_1$ band occurs at low energy, it is not observed in both the cases. Various spectral parameters like Dq, B and β have been calculated by using the values of $v_2$ and $v_3$ transitions, according to the equations of König (16). From the values of Dq and B, the energy of transition $v_1$ has been calculated (Table 3).

The observed electronic transitional energies are suggestive of octahedral geometry for these complexes. The B values for the complexes are lower than those for the free ion value, which is an indication of orbital overlap and delocalization of
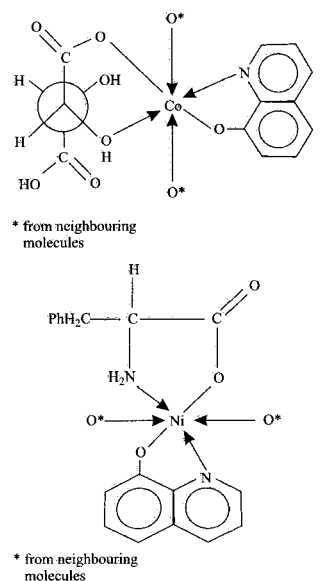


Figure 1. Proposed sructures and bonding for (a) Co(Q)(Tar)·H₂O and (b) Ni(Q)(Phe)·H₂O.

d–orbitals. The present β values are less than unity, suggesting an appreciable covalent character of the M–L bonds.

## Thermal studies

The TG and DTA experiments were conducted at a constant heating rate of 10°C per minute. The TGA of the complexes shows that they are thermally quite stable to a varying degree. The complexes show a gradual loss in weight due to decomposition by fragmentation with increasing temperature. The complexes [Co(Q)(Tar)]·H₂O and [Ni(Q)(Phe)]·H₂O show a loss in weight corresponding to one water molecule. The data on observed and calculated weight loss due to crystallization water is presented in Table 4. The complexes display an endothermic DTA peak in the range 117–127°C, which is attributed to the release of water molecule. As the temperature is raised, the DTA curve shows two strong exothermic peaks around 360 and 450°C, which may correspond to

Table 5. Biological activity (MIC μg/ml) data.

| Compound | Antibacterial activity | | | | | | Antifungal activity | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| [Co(Q)(Tar)]·H₂O | 100 | 100 | 200 | 100 | 200 | 100 | 150 | 200 |
| [Ni(Q)(Phe)]·H₂O | 500 | 450 | 500 | 200 | 250 | 200 | 200 | 200 |
| Tetracycline | 2.0 | 2.5 | 2.0 | 0.5 | 1.5 | 3.0 | – | – |
| Amphotericin | – | – | – | – | – | – | 1.5 | 1.0 |

1. *C. diphteriae*; 2. *E. coli*; 3. *S. typhi*; 4. *S. dysenteriae*; 5. *S. aureus*; 6. *V. cholerae*; 7. *C. albicans* and 8. *A. niger*.

the elimination of HQ and secondary ligand fragments, respectively.

On the basis of results of the physicochemical studies, the bonding and structure for the two metal complexes may be represented as shown in Figure 1.

## Biological activity

The antibacterial and antifungal activity of the ligands and the complexes were assayed against some of the bacteria and fungi. The cup–plate method was used to assay antibacterial activity against *C. diphtheria*, *E. coli*, *S. typhi*, *S. dysenteriae*, *S. aureus* and *V. cholerae*. The solvent used was dimethylformamide (DMF), and the sample concentration was 50–500 μg/ml. The results were compared against the controls, which were screened simultaneously. The activity was measured by measuring the diameter of the inhibited zone in millimeters (mm).

The results of the cup–plate method shown in Table 5 indicate the moderate activity of mixed ligand Co(II) complex against selected bacteria, while all of the selected microorganisms except *S. dysenteriae* and *S. aureus*, show general resistance to the Ni(II) complex. Thus, the Co(II) complex is more active than the Ni(II) complex, which may possibly be due to the low oxidation potential of Co(II). While coordination with Co(II) appears to enhance the activity, complexation with Ni(II) seems to have negative effect on the activity of parent ligands. The presence of bulky electron donating substituent may be responsible for the enhancement of such biological activity as observed in many cases (17). Compared to the standard compound tetracycline, the present complexes are much less active against the representative strains of microorganisms.

Table 6. Acute oral toxicity data.

| Compound | LD₅₀ (mg/kg b.w.) |
|---|---|
| [Co(Q)(Tar)]·H₂O | 1880 |
| [Ni(Q)(Phe)]·H₂O | 1505 |
| Tetracycline | 6443 |

Tube dilution method was used to assay antifungal activity against *C. albicans* and *A. niger*. The minimum inhibition concentrations of the two complexes against these fungi are recorded in Table 5. Both the complexes show antifungal activity to a moderate extent against the two fungi. Compared to the standard antifungal compound amphotericin, the present complexes are much less active against the representative strains.

The results suggest (18) that the chelation reduce considerably the polarity of the metal ions in the complexes, which in turn increases the hydrophobic character of the chelate and thus enables its permeation through the lipoid layer of microorganisms.

## Acute toxicity

Toxicity studies usually provide important basic information required for consideration of safe use of chemicals. Acute toxicity gives a rapid indication of the potential hazard and indicates the class of toxicity to which a chemical belongs. One of the basic tests for determining the relative acute toxicity in the animals is to find out LD₅₀.

Acute oral toxicity studies on [Co(Q)(Tar)]·H₂O and [Ni(Q)(Phe)]·H₂O were carried out in rats. The

patterns of symptoms found for both the compounds were similar but varied in degree and intensity with doses. The symptoms observed in animals after administering the different doses were: excitation, respiratory disturbances, tremors, convulsions and death. The $LD_{50}$ values are recorded in Table 6. As compared to Co(II) chloride ($LD_{50}$ value = 766 mg/kg) and Ni(II) chloride (105 mg/kg) the mixed ligand cobalt/nickel complexes show higher values, indicating the increase in $LD_{50}$ value due to complexation. Compared to the standard compound (19), the present complexes show more toxicity.

## CONCLUSIONS

Based on the above discussion and information available in the literature, the following conclusions may be drawn.

Higher decomposition temperature and electrical conductance studies show the presence of strong metal–ligand bonding and non–electrolytic nature of the complexes, respectively. Magnetic studies are indicative of octahedral geometry, which is confirmed by crystal field transitions shown by the electronic spectra. IR spectra show bonding of the metal ion through N/O and O of the two ligands and presence of crystallization water, confirmed by thermal analysis.

The studies on antimicrobial activity indicate that among other factors, constitution of the ligand, its coordination with the metal ion, the nature of metal ion, its oxidation state in the complex and the strain of the microorganism has influence on antimicrobial activity. Metal ions with low oxidation potential show more antibacterial activity. Coordination of bulky electron donating ligands to such metal ions enhances the activity. As compared to the Ni(II) complex, the mixed ligand Co(II) complex shows better antimicrobial activity against the selected strains. Compared to the standard antibacterial/antifungal compound, the present complexes are much less active against the representative strains. $LD_{50}$ values serve to provide a convenient way to classify the chemical into toxicity classes. The results suggest that both the complexes are moderately toxic.

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