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DNA binding and biological activity of mixed ligand complexes of Cu(II), Ni(II) and Co(II) with quinolones and N donor ligand

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Abstract: Mixed ligand complexes of Cu(II), Ni(II) and Co(II) have been synthesized by using levofloxacin and bipyridyl and characterized using spectral and analytical techniques. The binding behavior of the Ni(II) and Cu(II) complexes with hs-DNA were determined using electronic absorption titration, viscometric measurements and cyclic voltammetry measurements. The binding constants calculated for Cu(II) and Ni(II) complexes are 2.0 x 10^4 and 4.0 x 10^4 M^{-1} respectively. Detailed analysis reveals that the metal complexes interact with DNA through intercalative binding mode. The protective activity of Cu(II) and Ni(II) complexes with ct-DNA was carried out using agarose gel electrophoresis technique. The antioxidant activities for the synthesized complexes have been tested and the antibacterial activity for Ni(II) complex was also checked.

Key words: Intercalation, hypochromism, red shift, peak potential.

Introduction

Quinolones are large group of synthetic antibacterial agents containing 4-oxo-1,4-dihydroquinoline Skelton used in practice for treatment of a variety of bacterial infections[1-3]. Since the main targets of quinolones are DNA gyrase and topoisomerase IV participating in DNA replication[4], the interaction with DNA and the antibacterial activity are of great importance and thoroughly studied[5]. Levofloxacin (Figure 1) is used to treat infections including respiratory tract infections, cellulites, urinary tract infections, prostrates, anthrax, endocarditis, meningitis, pelvic inflammatory disease, traveler's diarrhea and Plague.

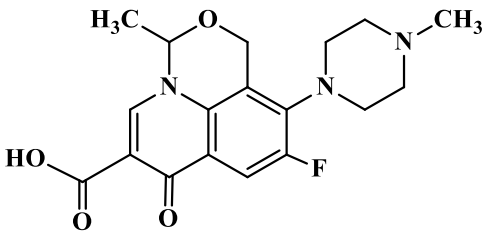

Figure 1. Structure of Levofloxacin

Based on the increased activity shown by metal-drugs complexes in comparison to their parent compounds[6], diverse complexes of metal and quinolone ligands have been synthesized and characterized. Their interaction with DNA[7-10] and serum albumin proteins[11-14], their antibacterial activity[15-17] and potential antitumor activity[18-20] have been evaluated in comparison to free quinolones. Mixed ligand of nickel and copper with quinolones using NN donor ligands have been synthesized and explored for their biological activities[21-23]. Nickel is one of the most essential elements to a healthy life for humans and higher animal species like chicken, rats, pigs, cows, sheep and goat[24-25]. It stabilizes DNA and RNA against thermal denaturation[24-25] and activates many enzymes like arginase, tyrosinase and phosphor-glucomutase. As for copper, the role of its compounds in the treatment of numerous chronic diseases is well established. Moreover, numerous metal compounds are able to act as anti-oxidants[26], antimicrobial[27], antiparasitic[28], anti-inflammatory, anticonvulsant29 and antitumor agents[30]. The chelation of metal (II) with ligand reduces the polarity of the metal ion and this, by the overlapping of the ligand orbital and the partial sharing of the metal ion positive charge with donor groups. The increase in the delocalization of π electrons over the whole ligand enhances the penetration of the complexes into the lipid membrane and the blocking of the metal binding sites on the enzymes of microorganisms[31]. The complexes may also disturb the respiration processes of the cell, block the protein synthesis and restrict further growth of the organism.

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Herein, we synthesized the mixed ligand complexes of Cu(II), Ni(II) and Co(II) with levofloxacin and bidentate bipyridyl ligands. The Cu(II) and Ni(II) complexes were tested for DNA interaction using UV-vis spectroscopy, cyclic voltammetry, viscosity and gel electrophoresis. The antioxidant activity for all the synthesized complexes was checked. The in vitro antimicrobial activity of Ni(II) was evaluated against different strains of bacteria.

Experimental studies:

Materials and Methods

All reagents, chemicals and solvents were of analytical grade and were used as such. Double distilled water was used throughout the experiment. Levofloxacin, NaCl, bipyridyl, DPPH, NBT, riboflavin, TBA, TCA, Tris and hs-DNA were purchased from Sigma Chemical Company. Cu(II), Ni(II) and Co(II) chlorides were purchased from E-Merck (India) Ltd.

DNA stock solution was prepared by diluting hs-DNA in tris buffer (containing 25 mM Tris HCl and 50 mM NaCl at pH 7.2) followed by the exhaustive stirring for three days, the solution was then kept at 4 $^{\circ}$C for no longer than a week. The stock solution of DNA gave a ratio of UV absorbance at 260 and 280 nm (A_{260}/A_{280}) of 1.88, indicating that the DNA was sufficiently free from protein contamination. The DNA concentration was determined by UV absorbance at 260 nm after 1:20 dilution using the following absorption coefficient ε=6600 cm^{-1} [6].

IR spectra of the complexes were recorded on a FTIR (Fourier transform infrared) spectrometer with samples prepared in KBr pellets. Electronic spectra were recorded on Shamidzu-UV-3600-UV-VIS-NIR spectrophotometer. The NMR spectra were obtained on Bruker DRX 400 spectrometer operating at room temperature. Magnetic measurements were carried out on magnetic susceptibility balance of Sherwood Scientific (Cambridge U.K.) at room temperature. Elemental analysis was performed on Perkin Elmer 2408 elemental analyzer. Molar conductance was measured at room temperature on Systronic conductivity bridge. Cyclic voltammetry was performed on SAS SP 150 Biologic Science Instruments carried out in 30 ml three electrode electrolytic cell. The working electrode was platinum disk, a separate Pt single sheet electrode was used as counter electrode and Ag/AgCl electrode saturated with KCl was used as reference electrode. KNO_3 and tris buffer were used as supporting electrolyte. The Cyclic voltammogram of the complex was recorded in tris HCl buffer (pH= 7.2) at 100 mV/s.

All electrochemical measurements were performed at room temperature. Hydrodynamic measurements were carried out from the observed flow time of hs-DNA containing solution (t > 100 seconds) corrected for flow time of the tris buffer alone (t_0) using ostwalds viscometer at 25 ± 0.01 $^{\circ}$C. Flow time was measured with a digital stop watch with least count of 0.01s.

Hydroxyl radicals generated by Fenton reaction were used to induce oxidative damage to DNA**.** The reaction mixture (15 µL) contained 25 mg of DNA in 20 mM of phosphate buffer saline (pH 7.4), 500 µg of test compounds were added and incubated with DNA for 15 minutes at room temperature. The oxidation was induced by treating DNA with 1 µL of 30mM of H_2O_2, 1 µL of 20 mM ferric nitrate and 1 µL of 100 mM ascorbic acid and incubated for 1 h at 37 ^{O}C. The reaction was terminated by the addition of loading dye (40% sucrose and 0.25% bromophenol blue) and the mixture was subjected to gel electrophoresis[32] using Hi Media LA666 in 0.7% agarose/TAE buffer run at 100 Volt. DNA was visualized by Gel Doc system.

To evaluate the antibacterial activity of Ni(II) complex**,** seven standard bacterial strains i.e. *Staphylococcus. aureus, Proteus vulgaris, Escheria coli, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumonia* were used. The antibacterial activity by agar disc diffusion assay was carried out as determined by Bauer et al[33]. Erythromycin was used as the reference antibacterial agent while 10% aqueous DMSO was used as vehicular control.

The antioxidant activity was carried out using different assays. In DPPH (2,2-Diphenyl-1-picrylhydrazyl Diphenyl-1-picrylhydrazyl) assay, quantitative measurement of radical scavenging properties of metal complexes were carried out according to the method by Blios et al[34]. Briefly 0.1 mM solution of DPPH was prepared in methanol and 1 mL of this solution was added to 3 mL of metal complex (100-300 µg/mL) and shikonin (300 µg/mL). α-tocopherol was used as a reference antioxidant. Discoloration of reaction mixture was measured at 517 nm after incubation for 30 minutes.

The Superoxide anion radical scavenging activity involves measurement of scavenging activity of all the metal complexes based on the method described by Liu et al[35] with slight modification. 100µL riboflavin solution (20µg), 200µL EDTA solution (12 mM), 200µL methanol and 100 µL nitrobluetetrazolium (NBT) solution (0.1 mg) were mixed in test tube and reaction mixture was diluted up to 3mL with phosphate buffer (50 mM). The absorbance of the solution was measured at 590 nm using phosphate buffer as blank after illumination for 5 minutes. Different concentrations (50 µl) i.e. 100 µg, 200 µg, 300 µg of complex solutions were used. Decreased absorbance of the reaction mixture indicates an increased super oxide anion scavenging activity.

In hydroxyl scavenging activity-deoxyribose assay[36], the colorimetric deoxyribose (TBARS) method was applied as the reference method of comparison for determining the hydroxyl radical scavenging activity of metal complexes. The reacting mixture for the deoxyribose assay contained in a final volume of 1mL the following reagents: 200 µL of KH_2PO_4-KOH (100 mM), 200 µL of

deoxyribose (15 mM), 200 µL of Ferric Chloride (500 µM), 100 µL of EDTA (1 mM), 100 µL of ascorbic acid (1mM), 100 µL of Hydrogen peroxide (10mM) and 100 µL of complex (100-300µg/mL). Reaction mixtures were incubated at 37 ºC for one hour. At the end of the incubation period, 1 mL of 1% (w/v) thiobarbituric acid (TBA) was added to each mixture followed by the 1 mL of 2.8% (w/v) trichloroacetic acid (TCA). The solutions were heated on a waterbath at 80 ºC for 20 minutes to develop pink coloured malonaldehyde-thiobarbituric acid (MDA-TBA) adduct and the absorbance of the resulting solution was measured at 532 nm.

In Ferric thiocyanate method (FTC)[37] 2 ml of complex solution (100-300 µg/mL) was mixed with 2.88 ml of linoleic acid (2.51%, v/v in 4 ml of 99.9% ethanol), 8 ml of phosphate buffer 0,05M at pH 7 and 3.9ml of distilled water. The whole reaction mixture was incubated at 40 ^{O}C for 96 h. To 100, 300 and 400 µL of this solution, 9.7, 9.4 and 9.3 mL of 75% (v/v) ethanol were added respectively followed by 0.1 mL of 30% ammonium thiocyanate to each one. Precisely after three minutes, 0.1 ml of 3.5% v/v HCl was added to the reaction mixtures, the absorbance at 500 nm of the resulting solutions was measured and recorded again after 24 h, until the absorbance of the control has reached the maximum value. α- tocopherol was used as reference antioxidant substance.

In thiobarbituric acid assay, thiobarbituric acid was added to the reaction mixture where it interacts with malanoaldhyde and the TBARS produced was measured spectrophotometrically[38]. To 2 mL of reaction mixture of ferricthiocyanate assay, 2 mL of TCA (20%) and 2 mL TBA (0.67%) were added, kept in boiling water for 10 minutes and later on, cooled under tap water. The reaction mixture was centrifuged at 3000 rpm for 20 minutes and the supernatant was read at 500 nm. α- tocopherol was used as reference antioxidant substance. The capacity to scavenge the radicals was calculated using the following equation:

$$\% \text{ inhibition} = A_c - A_s/A_c \times 100$$

Where 'A_c' is the absorbance of the control reaction (reaction mixture without any antioxidant substance) and 'A_s' is the absorbance of reaction mixture with reference substance or complex. The experiments were repeated thrice.

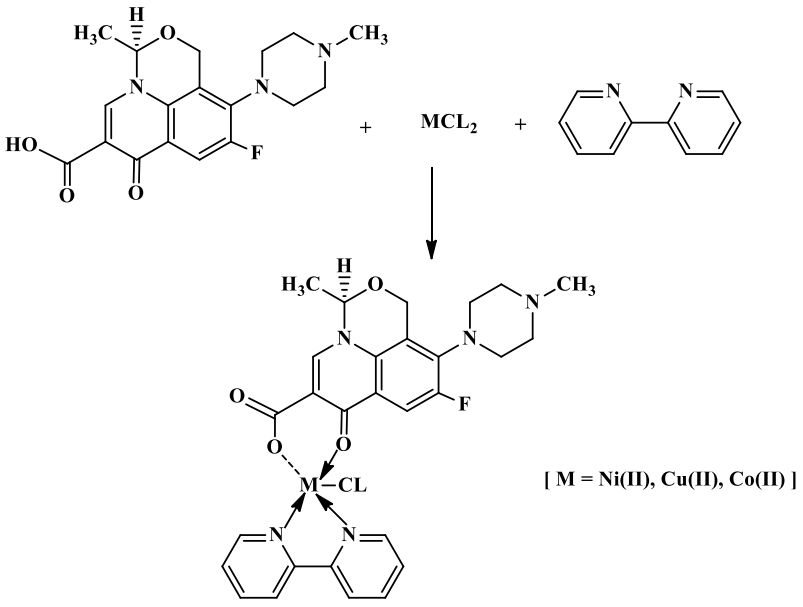
General synthesis of complexes

To a solution of Levofloxacin (3.61g, 10 mmol) and 2,2' bipyridyl (1.56g, 10 mmol) in 50 mL absolute ethanol was added Nickel(II) chloride in 1:1:1 molar ratio and refluxed for 10 minutes. A blue precipitate was obtained, isolated from the hot solution, washed with ether and dried in vacuo. Similar procedure was adopted for the preparation of Cu(II) and Co(II) complexes.

$C_{28}H_{27}N_5O_4FNiCl$: Yield 70%, m.p 350 ºC, Anal. (%Calcd./Found) C; 55.06/55.18, H; 4.45/4.49, N; 11.46/11.50. FT-IR (KBr, cm^{-1}); 1632 ν(C=O) pyridine, 1564 ν_{asym}(COO), 1365 ν_{sym}(COO), ^{1}H NMR (400MHZ, DMSO-d_6) δ(ppm) 7.7- 8.0(m), 2.4- 3.5(m), 4.3- 4.7(m), μ_{eff} = 2.57, soluble in water.

$C_{28}H_{27}N_5O_4FCuCl$, yield 82%, m.p 437º C, C; 54.63/54.35, H; 4.42/4.36, N; 11.37/11.28. FT-IR (KBr, cm^1); 1634 ν(C=O) pyridine, 1580 ν_{asym}(COO), 1382 ν_{sym}(COO), μ_{eff} = 1.86, soluble in ethanol.

$C_{28}H_{27}N_5O_4FCoCl$, yield 78%, m.p 393 ºC, C; 55.04/54.09, H; 4.45/4.48, N; 11.46/11.49. FT-IR (KBr, cm^{-1}); 1630 ν(C=O) pyridine, 1575 ν_{asym}(COO), 1375 ν_{sym}(COO), μ_{eff} = 3.24, soluble in DMF and DMSO.



Scheme 1. General reaction sequence for the synthesis of the studied complexes

Results and Discussion

IR spectroscopy

IR spectroscopy confirmed the deprotonation and binding mode of levofloxacin. In the IR spectra of the complexes, the disappearance of ν(H-O) stretching vibration of free quinolones (3010, br.) is indicative of deprotonation of carboxylate group on binding to metal ion. The shifting of $\nu(C=O)_p$ stretching vibration band in levofloxacin from 1708 to 1630-1634 cm^{-1} confirms the carbonyl oxygen of pyridine ring as the coordination site[31]. The characteristic ν(COO) asymmetric and symmetric vibrations as strong bands at 1624 and 1340 cm^{-1} also shift to 1564-1580 cm^{-1} and 1365-1382 cm^{-1} in the metal complexes. The unidentate nature of carboxylate group is confirmed by the frequency separation of 200 cm^{-1} ($\Delta = \nu(COO_{asym} - COO_{sym})$[31,39]. The data are further supported by the appearance of ν(M-O) at 510-515 cm^{-1} and ν(M-N) at 535-542 cm^{-1} [40].

Electronic Absorption Spectra

The UV-vis spectrum of the Ni(II) complex was measured at room temperature in the region of 250-800 nm which exhibits bands at 294, 305, 325 nm respectively (Figure 2a) and a broad band at 557 nm assigned to $^3B_{1(F)} \rightarrow {}^3E_{(F)}$ transition (Figure 2b). The λ_{max} values are consistent with pentacoordinate geometry around Ni(II) ion[41]. The electronic spectrum of Cu(II) complex measured in the range of 240-800 nm displays bands in the region of 250-800 nm and 370 – 382 nm, assigned to intraligand π – π* and LMCT transitions. However, the d-d transitions observed in the region 600-675 nm are typical of penta-coordinated Cu(II) complexes having distorted square pyramidal geometry (d_{xy}, $d_{yz} \rightarrow d_x^2 - d_y^2$) [42]. The electronic spectrum of Co(II) complex displays band at 350 nm assigned to LMCT transition and another weak band due to d-d transitions at 625nm.

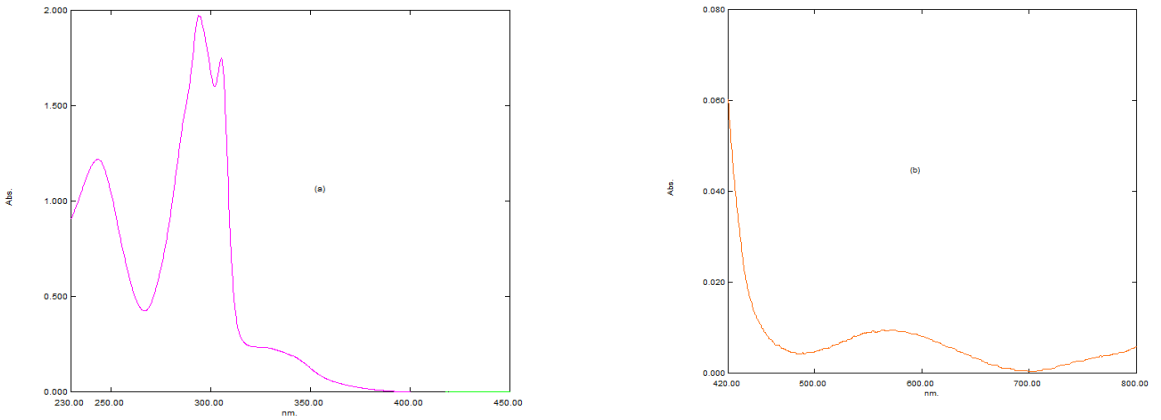


Figure 2. ***Electronic spectra of Ni(II) complex(a,b)***

EPR Spectrum

The X–band EPR Spectrum of Cu(II) complex was recorded at liquid nitrogen temperature (LNT) in solid state using tetracyanoethylene (g = 2.00278) as field marker. The EPR spectrum exhibited broad band having 'g' isotropic values of 2.09 characteristic of square pyramidal geometry. The spectral lines usually result from intermolecular spin exchange which broadens the spectral lines. The intermolecular spin exchange is caused by strong spin coupling, which occurs during collision of paramagnetic centers.

Magnetic susceptibility measurements

The magnetic susceptibility measurements for the Cu(II) complexes at room temperature lies in the range of 1.8-1.9 BM corresponding to one unpaired electron and are consistent with d^9 configuration around Cu(II) ion[43]. The magnetic measurements for Ni(II) complex show the magnetic value of 2.57 BM consistent with the pentacordinated geometry.

^{1}H NMR

The ^{1}H NMR spectra of the Ni(II) complex were recorded in DMSO-d_6 medium. The complex does not show any signal attributable to the carboxylic acid suggesting its involvement in the coordination of metal ion. The complex shows a broad multiplet at δ 7.7-8.0 ppm corresponding to aromatic protons and the peaks at δ 2.4-3.5 ppm are due to methyl protons. The methylene protons show peaks at δ 4.3-4.7 ppm.

DNA Binding Studies

UV-vis titration

Electronic absorption is an effective tool to examine the binding mode of complex with DNA[44-46]. Metal complexes bind to DNA via both covalent and non-covalent interactions[47]. Non-covalent interaction includes intercalation, binding to minor and major groove, sugar phosphate backbone and electrostatic binding mode[48]. Drugs binding with DNA via intercalation usually results in hypochromism and bathochromism of absorption band due to strong interaction between the aromatic chromophore of the molecule and base pairs of the DNA. On the other hand, absorption intensities of drugs are increased (hyperchromism) upon increase of the DNA concentration due to damage of the DNA double helix structure. The extent of hyperchromism is indicative of the partial or non-intercalative binding modes such as electrostatic and van der Waals interaction, hydrogen bonds and hydrophobic interaction.

The interaction of Ni(II) complex in water with hs-DNA was investigated through the change of absorbance at 256 nm with increasing concentration of hs-DNA. The spectra clearly show that bands at 256 exhibit hypochromism with red shift of 16 nm (Figure 3a). The bands at 294 nm and 305 nm completely disappear but the band at 325 nm displays a hyperchromism with blue shift of 20 nm to 305 nm (Figure 3b). These changes are typical of complexes bound to DNA through non-covalent interaction [49]. The red shift in absorbance are accompanied by increase in molar absorptivity so that isobestic points are formed at 285 nm indicating single mode of binding. The spectral features suggest that the Ni(II) complex bind to hs-DNA by intercalative binding. The observed hypochromism could be attributed to the contraction of hs-DNA helix axes as well as its conformational changes. The hyperchromism could be the result of secondary damage of hs-DNA double helix structure[44,50]. The carbonyl group in the Ni(II) complex could form hydrogen bonds with suitable donors like N_7 and O_6 of adjacent guanine bases of hs-DNA supported by hydrophobic interaction of pyridyl ring on surface of hs-DNA contributing to hyperchromism. To quantify the extent of binding of the complex with hs-DNA, the intrinsic binding constant K_b was calculated using the following equation

$$\mathbf{[DNA]/[\varepsilon_a-\varepsilon_f) = [DNA]/\varepsilon_b-\varepsilon_f+1/k_b(\varepsilon_b-\varepsilon_f)}$$

Where εa, εb and ε_f are apparent, bound and free extinction coefficient respectively. Fit the plot of $[DNA]/[\varepsilon_a-\varepsilon_f)$ *vs* [DNA], the K_b was obtained from the ratio of slope to intercept. The binding constant of the complex is 4.0 x 10^4 M^{-1}(Figure 4). From the binding constant, it is clear that the complex binds strongly to hs-DNA, however the binding constant is lower than the classical intercalators[47,48].

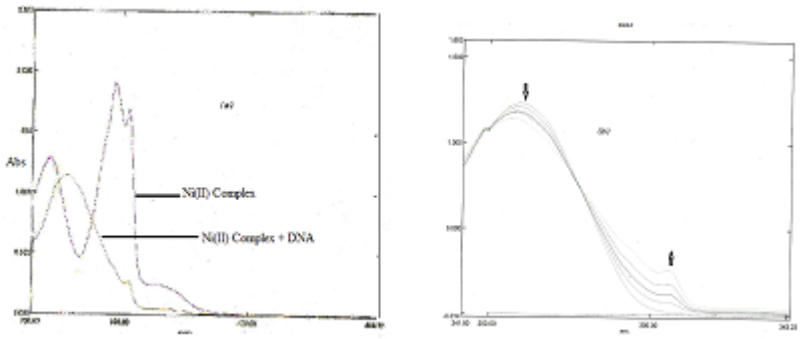


Figure 3. (a). Shift in absorption band of Ni(II) complex after addition of hs-DNA. (b). Electronic spectra of Ni(II) complex on addition of increasing amounts of hs-DNA

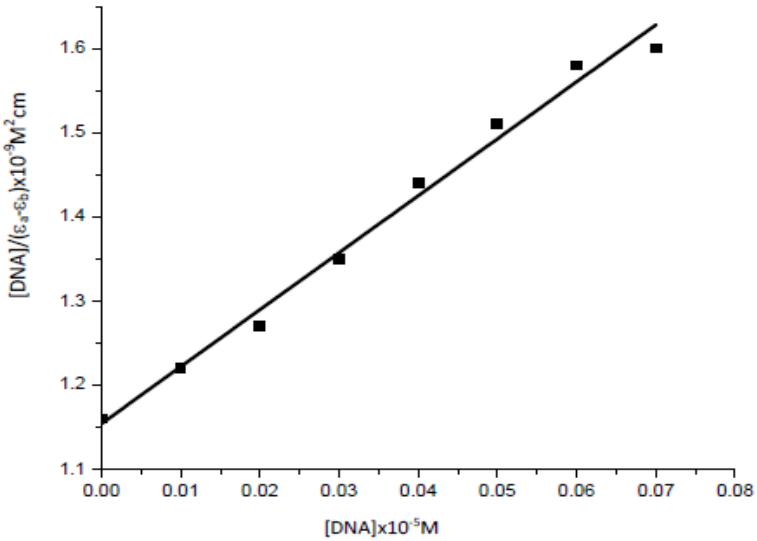


Figure 4. Plot of $[DNA]/(\varepsilon_a-\varepsilon_b)$ x10^{-9} M^2 cm vs [DNA] x 10^{-5}M for titration of hs-DNA with Ni(II) complex.

The interaction of Cu(II) complex with hs-DNA was also carried out. The absorption spectra of the Cu(II) complex in the presence and absence of hs-DNA shows red shift of 4 nm and hypochromism (Figure 5) revealing that the complex binds to hs-DNA by intercalation[51]. This may be due to the fact that π orbital of the base pairs of hs-DNA couples with the π orbitals of the ligand leading to bathochromism. Further, the π orbital is partially occupied by electrons thereby decreasing the transitional probabilities and resulting in hypochromism. The binding constant calculated for Cu(II) complex is 2.0 x10^4 M^{-1}.

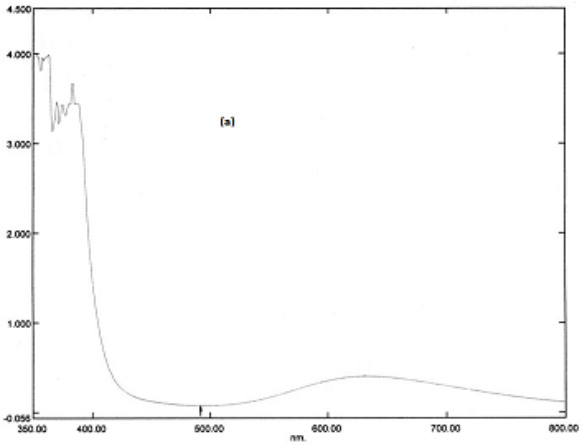


Figure 5(a). Electronic spectra of copper(II) complex

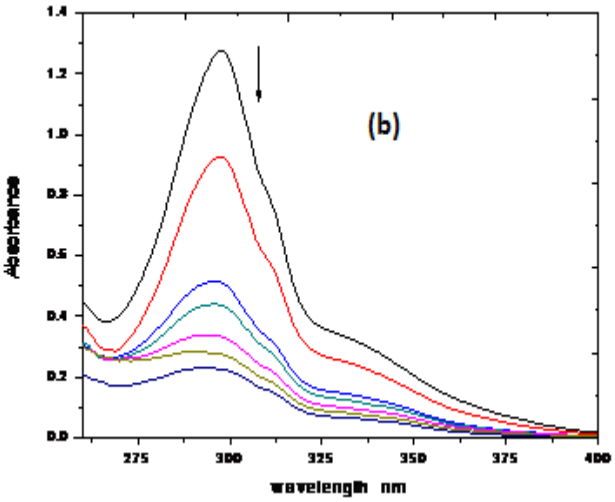


Figure 5(b). Electronic spectra of Cu(II) complex in presence of increasing amounts of hs-DNA.

Electrochemical studies

Cyclic voltammetry is widely used as simple and rapid method to study DNA interaction with metal complex. The cyclic voltammetry of Cu(II) complex alone and with hs-DNA were recorded in the range of -0.8 to 0.5 V with a scan rate of 100 mv/s in tris buffer (pH= 7.2). The cyclic voltammogram for Cu(II) complex exhibits one electron redox process involving Cu(II)/Cu(I) couple. The cathodic peak potential (E_{pc}) appears at -0.558V with I_{pc} at 4.466 mA. The addition of 10^{-3} M hs-DNA to Cu(II) complex causes a decrease in cathodic current (0.296 mA). Further, the peak potential E_{pc} shifts to a more positive potential (-0.450V). The observed decrease in current may be attributed to slow diffusion of an equilibrium mixture of free and DNA bound Cu(II) complex to the electrode surface[52]. Bard and co-workers[53] have discussed the binding modes between small molecules and DNA, If the interaction is through electrostatic binding mode, the formal potential shifts to a more negative potential while intercalative binding mode results in a more positive potential. In presence of hs-DNA, the Cu(II) complex shows positive shift revealing an intercalative binding mode between Cu(II) complex and hs-DNA base pairs (Figure 6)

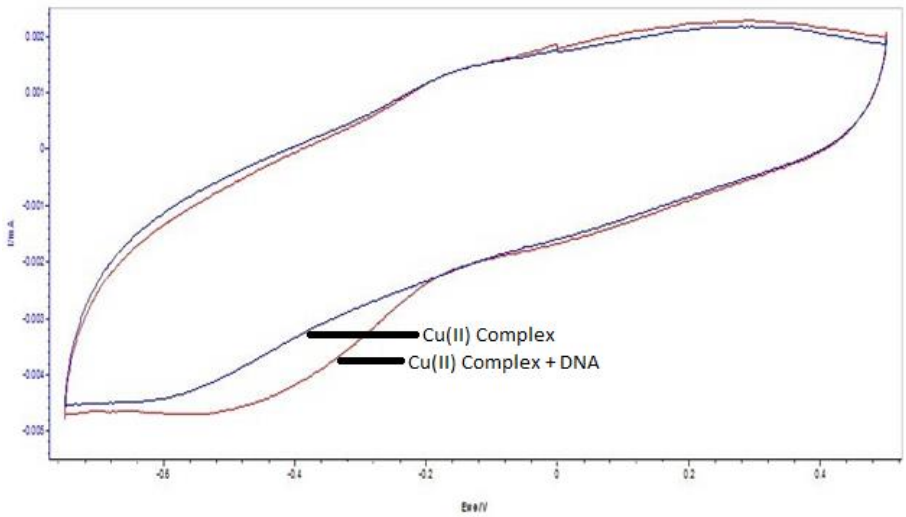


Figure 6. ***Cyclic voltammogram of Cu(II) complex at scan rate of 100 mV/s in the presence and absence of hs-DNA.***

Hydrodynamic measurements

A hydrodynamic method such as viscosity which is sensitive to changes in DNA length offers a least ambiguous and definitive method to determine the binding mode of DNA binding agents[54]. A classical intercalation binding demands that DNA helix must lengthen as base pairs are separated to accommodate the binding ligand leading to an increase in DNA viscosity[55]. There is little effect on viscosity if electrostatic or groove binding occurs[56]. Intercalating agents are expected to destabilize base pairs, causing lengthening of the double helix resulting in the increase of viscosity of DNA, while non-classical intercalators or groove binding of the complex could bend or kink the DNA helix reducing its effective length and concomitantly its viscosity[57]. The relative viscosity was calculated using the equation $t\text{-}t_0/t_0$, where t_0 is the flow time for the used buffer and t is the observed flow time for DNA in absence and presence of the complex. The results were presented as η/η_0 *vs* r (binding ratio), where r = [complex]/[DNA], η is the viscosity of hs-DNA in presence of the complex and η_0 is the viscosity of hs-DNA alone. The effect of Ni(II) complex on relative viscosity of hs-DNA by varying the concentration of complex shows pronounced increase. The Cu(II) complex also experiences an increase in viscosity of hs-DNA. The results reveal that metal complexes bind to hs-DNA *via* intercalation. The results obtained from the viscosity studies validate data obtained from uv-vis and electrochemical titration.

Gel electrophoresis

The ability of the complex to interact with DNA for protection from hydroxyl radicals is depicted in figure 7. The principle of the method is that molecules migrate in the gel as a function of their mass, charge and shape. We observed that Cu(II) and Ni(II) complexes show considerable DNA damage protective effect. The reference substance BHT used show protective effect. BHT is a known antioxidant substance, as it has the property of scavenging the free radicals. In Lane 2 reaction mixture was added to ct-DNA and was kept as negative control as no protective/antioxidant substance was used in this lane and as seen in Lane 2, radicals generated completely inhibit the DNA. However in Lane 3 only DNA was used as acting as positive control.

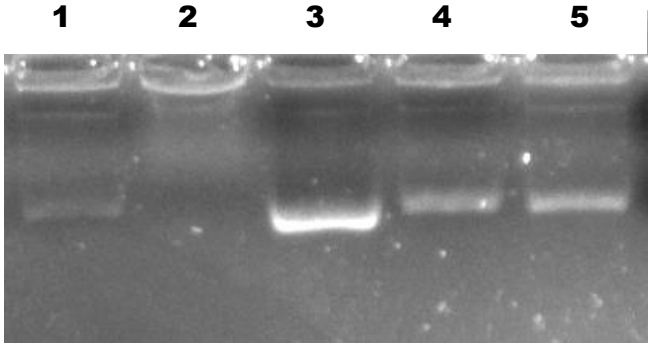


Figure 7: ***Protective DNA damage by levofloxacin- bipyridyl complexes.***

Lane 1. BHT+ Reaction mixture +ct-DNA Lane **2**. ct-DNA + Reaction mixture
Lane 3. ct-DNA alone **Lane 4**. Ni(II) complex (500 µg) + Reaction mixture + ct-DNA **Lane 5**. Cu(II) complex (500 µg) + Reaction mixture + ct-DNA .

Biological Activity

Antibacterial activity

The Ni(II) complex exhibits significant inhibition on the tested pathogenic strains. 10% aq. DMSO was used as negative control and showed no activity against all strains. The highest inhibition (36 mm diameter) was observed at high concentration of Ni(II) complex (200 µg) on *B. subtilis* with respect to the other strains and to erythromycin (25 mm) used as a positive control. The activity of Ni(II) complex was concentration dependent and the inhibition diameter of strains increases with the increase in Ni(II) complex concentration with an inhibition zone between 28-36 mm. In another experimental setup, the minimum inhibitory concentration (MIC) of the Ni(II) complex (Table 2) against the tested strains was determined. The MIC of Ni(II) complex was reported against *Staphylococcus aureus* (10 µg/ml) followed by *E.coli* (12 µg/ml) as compared to the studied strains.

Table 1 Antibacterial activity of Ni(II) complex against pathogenic bacterial strains

	Zone of inhibition mm				
Bacterial Strain	**200µg**	**100µg**	**50µg**	**25µg**	**Erythromycin (25mcg/disc)**
Proteus vulgaris	29 ± 1.32	26 ± 2.15	24 ± 3.45	20 ± 2.12	25 ± 1.17
Bacillus subtilis	36 ± 1.69	29 ± 1.56	26 ± 2.56	25 ± 2.72	29 ± 3.09
Staphylococcus aureus	30 ± 1.09	23 ± 1.97	21 ± 0.32	21 ± 3.42	22 ± 1.05
Escherichia coli	31 ± 2.17	26 ± 3.32	25 ± 1.71	22 ± 4.12	28 ± 1.91
Klebsiella pneumonia	28 ± 1.08	24 ± 2.12	23 ± 0.32	21 ± 3.12	ND
Psuedomonas aeruginosa	30 ± 1.0	26 ± 1.12	24 ± 0.39	22 ± 1.03	25 ± 2.03

Values are represented as mean ± Sd (*n* = 3), 10% aqueous DMSO used as negative control showed no activity

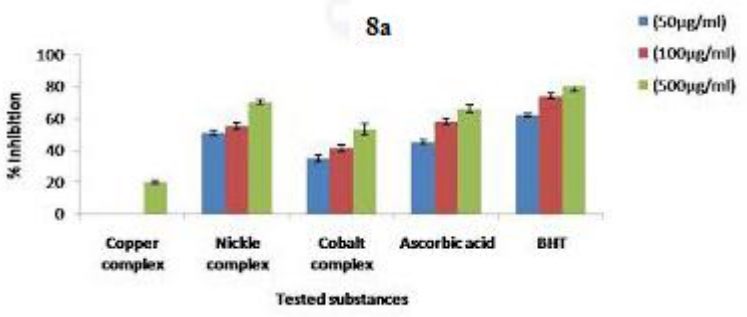
Table 2: MIC determination of Ni(II) complex against tested pathogenic bacterial strains

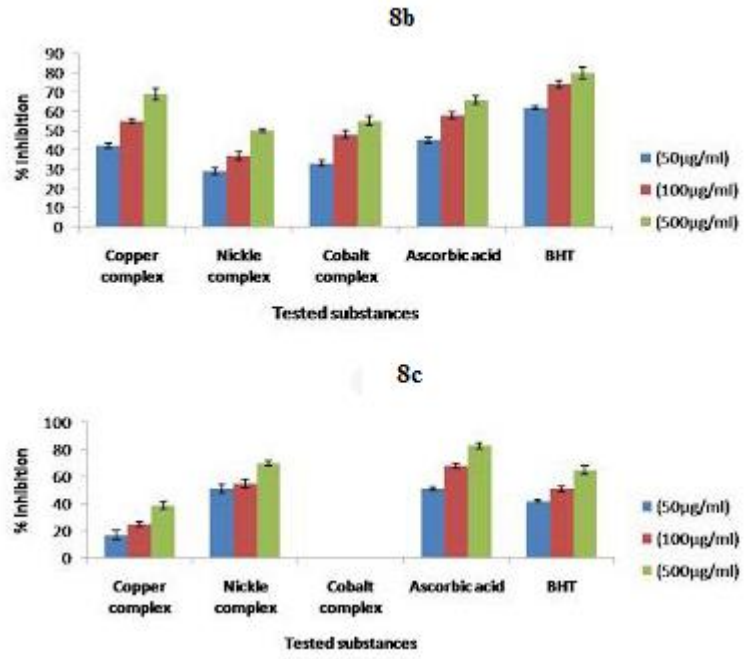
	MIC (µg/mL)	
Bacterial Strain	**Ni(II) complex**	**Erythromycin**
Proteus vulgaris	20 ± 2.41	18 ± 2.08
Bacillus subtilis	18 ± 2.327	24 ± 4.02
Staphylococcus aureus	10 ± 1.43	16 ± 2.17
Escherichia coli	12 ± 1.06	14 ± 3.95
Klebsiella pneumonia	24 ± 3.49	-
Psuedomonas aeruginosa	-	16 ± 1.49

Values are represented as mean ± SD (*n* = 3). The concentrations of the tested substances were in the range (24, 22, 20, 18, 16, 14, 12, 10, 7.5 µg/ml*)*. 10% aqueous DMSO (was) used as negative control showed no inhibitory activity.

Antioxidant activity

The antioxidant activity of the metal complexes was compared with a positive control (BHT) and Ascorbic acid, which is known to protect tissues and cells against various oxidative stresses[58]. The mechanism of scavenging of radicals cannot be evaluated by a single method; therefore, five different antioxidant models were used in this study[59]. The antioxidant activity of metal complexes in DPPH method was determined through the decrease in absorption strength by radical scavenging activities of Cu(II), Ni(II), Co(II) complexes, Ascorbic acid, and BHT at 500 µg/mL and were found to be 20%, 70%, 53%, 66% and 80% respectively (Figure 8a). We found that the free radical scavenging activity of Ni(II) complex was stronger than Cu(II) and Co(II) complexes. However BHT was found to have the highest scavenging activity. The activity of all the complexes were found to be concentration dependent.



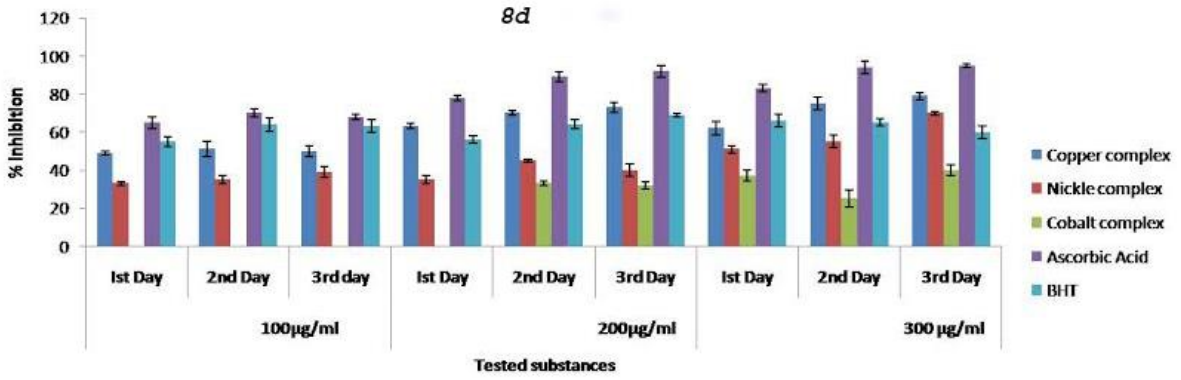


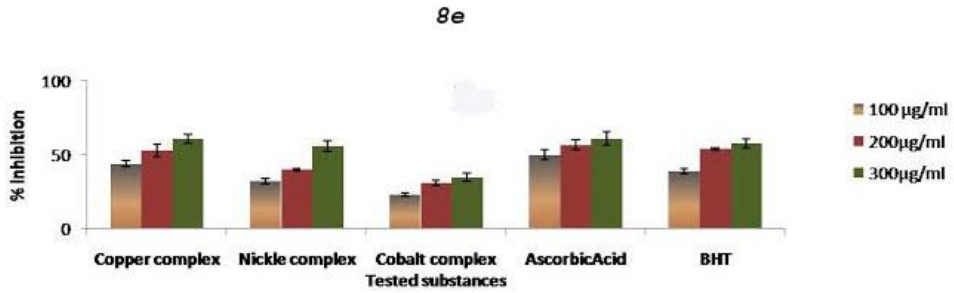
All the complexes exhibited moderate to high superoxide dismutase activity at variable concentrations. The Cu(II) complex exhibited higher scavenging activity (69%) followed by Co(II) and Ni(II) complexes at 500 µg/mL(Figure 8b).

The hydroxyl radicals are known to cause DNA damage by degradation of deoxyribose moiety which contributes to carcinogenesis, mutagenesis and cytotoxicity[60], however, the scavenging or chelation of radicals by any substance is due to the antioxidant capacity of that particular substance[61]. In our study, Ni (II) complex exhibited the highest chelating activity of hydroxyl radicals at 500 µg/ml. The scavenging activity decreased in the following order: Ascorbic acid (83%) > Ni(II) (70%) > BHT (65%)> Cu(II) (39%) at 500 µg/ml (Figure 8c).

FTC evaluates the effect of a reference antioxidant and metal complexes on preventing peroxidation of polyunsaturated fatty acids and linoleic acid. The % of inhibition was recorded after every 24 h and results were calculated for three consecutive days. The percentage of protective effect of linoleic acid peroxidation was 80% for Cu(II) complex, 70% for Ni(II) complex and 40% for Co(II) complex at 300 µg/ml concentration (Figure 8d).

In the TBA method, formation of malonaldehyde is the basis for evaluating the extent of lipid peroxidation. At low pH and high temperature malonaldehyde, the end product of lipid peroxidation, binds TBA to form a red colored complex. The concentrations used were 100– 300 µg/mL. The FTC method measures the amount of peroxide produced during the initial stage of lipid peroxidation. Subsequently at later stages of oxidation, peroxides decompose to form carbonyl compounds that are measured by the TBA method. Thiobarbituric acid assay is determined in the reaction mixture from the assay of FTC while in TBA assay, inhibitory activity of peroxide radicals is determined from the residual mixture earlier used in FTC method. At 300 µg/mL, the Cu(II) complex exhibited 61% inhibition of radicals, followed by Ni(II) complex (56%) (Figure 8e). Some of the reports mentioned the ferric reducing power of bioactive compounds such as phenolic substances and flavonoids[62-64]. The findings of this work confirm that the metal complexes can be used as an alternative therapy to combat various indigenous as well as exogenous stresses.





The inhibitor concentration for scavenging of radicals (IC_{50} µg/mL) was determined for all the antioxidant methods. From the results, it was observed that IC_{50} values varied with the complexes as well as with the type of method. The Cu(II) complex showed lowest value of 72 µg/mL in scavenging of superoxide radicals followed by Ni(II) complex with 95 µg/mL (Table 3). The Co(II) complex showed the comparatively higher value of IC_{50} in all the methods employed.

Table 3. IC_{50} determination of metal complexes

	IC_{50} µg/mL				
Complexes	**DPPH assay**	**SOD**	**Hyroxyl scavenging assay**	**FTC**	**TBA**
$C_{28}H_{27}N_5O_4FNiCl$	140	150	95	240	260
$C_{28}H_{27}N_5O_4FCoCl$	320	120	-	>300	-
$C_{28}H_{27}N_5O_4FCuCl$	>350	72	>300	100	140
Ascorbic acid	175	60	97	70	100
BHT	117	40	193	70	160

Conclusion

In this work, mixed ligand complexes of Cu(II), Ni(II) and Co(II) have been synthesized and characterized by various physicochemical studies with an aim to develop robust therapeutic agents. Complexes possess pentacoordinate geometry. The results reveal that both Ni(II) and Cu(II) complexes interact with DNA showing strong intercalation. The results of gel electrophoresis prove the cu(II) and Ni(II) complexes show considerable DNA damage protective effect. The MIC shows lower values for most of the tested strains respect to control confirming antimicrobial activity. All synthesized complexes show considerable antioxidant activity. Overall the mixed ligand complexes derived from levofloxacin and bipyridyl possess strong potential to be used as possible therapeutic agents.

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