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SPECTRAL CHARACTERIZATION DNA - BINDING STUDIES AND BIOLOGICAL ACTIVITY OF CR(III), CO(II), NI(II), HG(II) AND CD(II) COMPLEXES WITH NICOTINIC ACID HYDRAZIDE AND THIOCYANATE ION AS LIGANDS

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ABSTRACT

The complexes of Cr(III) , Co(II), Ni(II), Hg(II), Cd(II) were synthesized with the ligands Nicotinicacidhydrazide and (SCN-) and characterized by using Elemental analysis, Molar conductance measurements, IR, NMR, spectroscopic studies,all the complexes are identified as bidentate forms chelate complexes.Gel electrophoresis study reveals the fact that the copper complex cleaves super coiled pBR 322 DNA to nickel and linear forms in the presence and absence of nicotinic acid hydrazide. Antibacterial and antifungal activities of the complexes were studied and the complexes were screened against bacteria and fungi. The fungal activity data shows that the metal complexes are potent active than the parent ligand NHA.

KEY WORDS: **Nicotinicacidhydrazide, antifungal, thiocynate ion, DNA**

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INTRODUCTION

Heterocyclic compounds play a significant role in many biological systems, especially N-donor ligand systems being a component of several vitamins and drugs such as NHA[1]. Pyridines derivative are very important in biological activities such as anti-tubercular, anthelmintic, fungicidal, antitumor and antibacterial activities.[2-4] NHA acts as bidentate ligand sometimes it will act as monodentate ligand and having good ligating character, enhanced biological properties.

MATERIALS AND METHODS

All the chemicals used for the preparation of the ligands were Alfa Aesar quality and AR grade. Molar conductance of the complexes were measured using a Systronic conductivity bridge at room temperature in DMSO. Conductivity measurements ($\Omega^{-1}cm^2$ mol^{-1}) were carried out in DMSO using a Tacussel conductivity bridge model. Perkin-Elmer PE 938 spectrophotometers were used to record the IR spectra using KBr pellets. The antimicrobial screening studies were carried out at micro labs, Arcot, India.The buffer solution (50 mM NaCl-5 mM Tris–HCl) was used as the supporting electrolyte. Agarose gel electrophoresis method was carried out at micro labs, Arcot, India. Water purified using a Milli-Q system was used for all the present studies.

(i) Synthesis of Metal Complexes

The chromium, cobalt, nickel, mercury and cadmium complexes were synthesized by mixing 0.71g, 1.31g, 1.31gand 0.69g and 0.61g of nicotinic Hydrazide (3.79mmol, 6.93mmol, 6.93mmol, 3.64mmol and 3.21mmol) in methanol and the metal nitrates 1g $[Cr(NO_3)_3.9H_2O]$ 2.5mmol ;1g $[Co(NO_3)_2.6H_2O]$ 3.4mol; 1g $[Ni(NO_3)_2.6H_2O]$ 3.4mmol, 1g $HgCl_2$ 3.62 mmol1g $[Cd(NO_3)_2.4H_2O]$ 3.22 mmol in methanol. The mixture was heated in a microwave oven for about 10 seconds. Then 0.45g, 0.48g, 0.48g, 0.51g and 0.45g of sodium nitrite 6.40mmol, 6.9mmol, 6.9mmol and 7.30mmol and 6.40mmol in ethanol was added and the whole mixture was heated in a microwave oven for about 10 seconds. The precipitated complexes were filtered and washed with ethanol and dried. The elemental analysis values are in good agreement with the formulae of the complexes. The electrical conductivity values show the non electrolytic nature of the complexes. It is represented in Table-1. The ligand (L) is soluble in common organic solvents such as THF, C_2H_5OH, CH_2Cl_2 and DMSO. The octahedral metal complexes are highly soluble in DMSO and DMF and slightly soluble in CH_2Cl_2 and $CHCl_3$

(ii) DNA Binding activity

The DNA binding experiments were performed at 30.0 ± 120.2oC. The DNA concentration per nucleotide was determined by electronic absorption spectroscopy using the known molar extinction coefficient value of 6600 M-1 cm-1 at 260nm [11]. Absorption titration experiments of copper(II) complex samples in buffer solution (50 mM NaCl-5 mM Tris–HCl, pH 7.2) were performed by using a fixed complex concentration to which increments of the DNA stock solutions were added. Copper(II) complex–DNA solutions were allowed to incubate for 10 minutes before the absorption study was carried out. For fluorescence-quenching experiments, DNA was pre-treated with ethidium bromide (EB) for 30 minutes. The copper(II) complex samples were then added to this mixture and their effect on the emission intensity was measured. Samples were excited at 450 nm and emission was observed between 500 nm and 800 nm. Viscosity measurements were carried out using an Ubbelodhe viscometer maintained at a constant temperature of 30.0±0.1 °C in a thermostatic water-bath. Calf-thymus DNA samples approximately 200 base pairs in average length were prepared by sonicating in order to minimize complexities arising from DNA flexibility [12]. Flow time was measured with a digital stopwatch and each sample was measured three times and an average flow time was calculated. Data were presented as $(\eta/\eta 0)1/3$ versus binding ratio, where η is the viscosity of CT DNA in the presence of complex, and $\eta 0$ is the viscosity of CT DNA alone.

(iii)DNA Cleavage

For the gel electrophoresis study, super coiled pBR322 DNA (0.1 µg) was treated with the copper(II) complex in 50 mM Tris–HCl-18 mM NaCl buffer, pH 7.2. The samples were electrophoresed for 3 hours at 50 V on a 0.8 % agarose gel in tris–acetic acid–EDTA buffer. The gel was stained with 0.5 µg of ethidium bromide and photographed under UV light. 2.5

(iv) Microbial Assay

Antimicrobial analysis was followed using standard agar well diffusion method to study the antimicrobial activity of essential oils. Each bacterial and fungal isolate were suspended in Brain Heart Infusion (BHI) broth and diluted to approximately 105 colony forming unit (CFU) per mL. They were flood inoculated onto the surface of BHI agar and then dried. 5mm diameter wells were cut from agar using a sterile cork-borer and 30 µL (5µg compound in 500µL DMSO) of the sample solution were poured into the wells. The plates were incubated for 18 hours at 37oC for bacteria and at room temperature for fungi. Antimicrobial activity was evaluated by measuring the zone of inhibition in mm against the test microorganisms. DMSO was used as solvent control. Ciprofloxacin was used as a reference antibacterial agent. Ketoconazole was used as a reference antifungal agent. The tests were carried out in triplicates.

RESULTS AND DISCUSSION

The prepared complexes Cr(III) , Co(II), Ni(II), Hg(II), Cd(II) are coloured but the Cadmium complexes are colourless (table-1). All are soluble in DMSO and DMF. The molar conductance values obtained for these complexes at the concentration of 10^{-3} M are in the range of 10-20 Ω^{-1} Mol^{-1} cm^2. These values are too low to account for any dissociation of the complexes in DMF. Hence these complexes can be regarded non-electrolytes[5-6]. In IR spectrum the aromatic C-H stretching frequency found at 3408cm^{-1} which gets shifted in 3281-3433cm^{-1} in complexes. The –C=O group frequency in NHA at 1611cm-1 shifted to 1601 to 1643 cm-1 in complexes[6]. The -C=N frequency in pyridine ring of NHA and its complexes are found in 1369-1550 cm^{-1}. In all the complexes the asymmetric stretching frequencies nitrite ion will appear between 1363-1381 cm-1 in all the complexes and the symmetric stretching frequencies of nitrite ion at 1340cm^{-1}.

1. H-NMR Spectrum

In the ^{1}HNMR spectrum of NHA the δ value at 7.44 to 7.48 corresponds to the aromatic proton which is almost same in mercury complex. The $–NH_2$ value is at 3.56ppm in NHA and 3.56 in mercury complex[7-8]. V_{M-ONO} values are lying in 620-826 cm^{-1} and V_{M-N} values are lying in 820-840 which shows the metal ligating ability9-10.

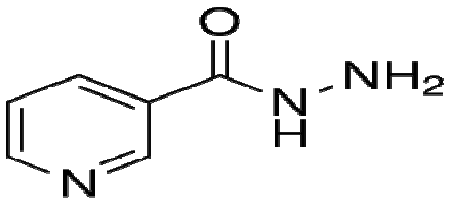

Figure 1
Structure of NHA

Table 1
Elemental Analysis, Magnetic Moments, Molar Conductance of the Complex.

S No.	Complex	Colour	Conductance (ohm^{-1} cm^2mol^{-1})	Yield %	C %	H %	N %	Metal %
1.	$[Cr_2(NHA)_2(NO_2)_6]$	Magenta	101.7	69.5	28.14 (28.12)	2.73 (2.69)	49.25 (49.21)	13.55 (13.50)
2.	$[Co(NHA)_2(NO_2)_2]$	Orange red	85.2	79.13	40.26 (40.25)	3.35 (3.34)	26.84 (26.83)	14.12 (14.11)
3.	$[Ni_2(NHA)_2(NO_2)_2]$	Pale Blue	95.7	73.18	33.88 (33.86)	3.29 (3.27)	26.35 (26.33)	13.81 (13.80)
4.	$[Hg(NHA)(NO_2)_2]$	Pale yellow	69.9	78.21	24.59 (24.57)	2.39 (2.37)	23.91 (23.89)	21.71 (21.70)
5.	$[Cd(NHA)(NO_2)_2]$	Pale white	79.0	77. 0	21.08 (21.05)	2.04 (2.02)	20.49 (20.47)	32.91 (32.90)

Table 2
IR Spectral data of NHA and its metal complexes (Cm^{-1})

Complex	$V_{C=C}$	$V_{C=O}$	$V_{C=N}$	V_{C-N}	V_{M-N}	V_{M-ONO}
NHA	2045	2100	2080	1850	-	-
$[Cr_2(NHA)_2(NO_2)_6]$	2047	2100	2000	1853	832	620
$[Co(NHA)_2(NO_2)_2]$	2043	2102	2020	1862	828	622
$[Ni_2(NHA)_2(NO_2)_2]$	2044	2102	2010	1867	830	826
$[Hg(NHA)(NO_2)_2]$	2043	2100	2100	1060	840	630
$[Cd(NHA)(NO_2)_2]$	2044	2110	2005	1875	835	622

2. Powder X-ray Analysis

The XRD (powder pattern) of the complexes $[Co(NHA)(NO_2)_2]$ and $[Ni(NHA)_2 (NO)_2]$ were indexed in X-ray difractometer and the unit cell parameters have been calculated with the help of a computer from 2 θ values (Fig.1). The direct constant parameters like A, B, C, q, p, y, nd v (volume) are given in Table-3[9].

Table-3
X-ray powder pattern reports

Compound	2θ			Unit Cell Parameters	Density	N	Possible Geometry
$[Co(NHA)(NO_2)_2]$	13.284	13.936	16.141		(gcc) 0.83	1	Monoclinic
	17.228	18.130	18.264	A = 13.300 Å			
	19.300	21.756	22.659	B = 20.543 Å			
	25.010	25.516	30.914	C = 6.985 Å			
	32.051	39.253	41.659	□ = 90.000°			
	42.244	43.849	46.389	□ = 102.325°			
	47.308	48.194	49.881	γ = 90.000°			
	50.032	51.419	53.174	V = 1864.37			
	53.541	53.976	54.076	$Å^3$			
	54.277	55.680	56.148				

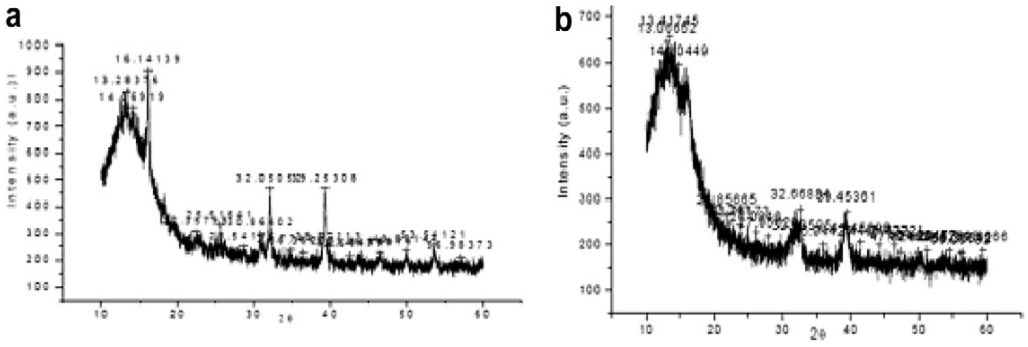

Figure 2
XRD (Powder Pattern) of the complexes (a) [Co (NHA) $(No_2)_2$] and (b) [Ni $(NHA)_2(No)_2$].

3. Antibacterial Activity

For in- vitro antimicrobial activity, the investigated compounds were tested against the bacteria such as Shigella dysenteriae, E. coli and Bacillus subtilis. The minimum inhibitory concentration (MIC) values of the compounds against the growth of microorganisms are summarised in Table 2. It is observed that the copper and cadmium complexes are more active in Pseudo- Mona aeruginosa and B. subtilis, respectively compared to other bacterial organisms. Nickel and cobalt complexes are moderately active in all bacterial organisms compared with standard streptomycin. From the results, it is found that the copper complex is more active in Streptococcus-b-haemolyticus than the other complexes.

4. Antifungal Activity Studies

The results of the antifungal screening of the nicotinamide and the metal complexes with Candida albicans, A. niger and Asper- gillus fumigates at concentration of 200 lg by disc method are given in the Fig. 6. Comparative studies of the ligands and their complexes indicated that metal complexes exhibit higher antifungal activity than the free ligands (Table 8). The anti-fungal activity results revealed that the ligands and their Cu(II), Co(II) and Ni(II), complexes have exhibited weak to good activity against A. niger and A. flavus. The ligand and its Cu(II) and Co(II) complexes show weak activity when compared to the standard drug clotrimazole. The order of the metal complexes follows Cu(II) > Cd(II) > Ni(II) > Co(III) > Mn(II) > Fe(III) > Cr(III). The higher activity of metal complexes can be explained on the basis of overton's concept and chelation theory. According to overtons concept of cell permeability, the lipid membranes that surround the cell favour the passage of only the lipid soluble material due to which lip solubility is an important factor, which controls antimicrobial activity. On chelation, the polarity of metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive

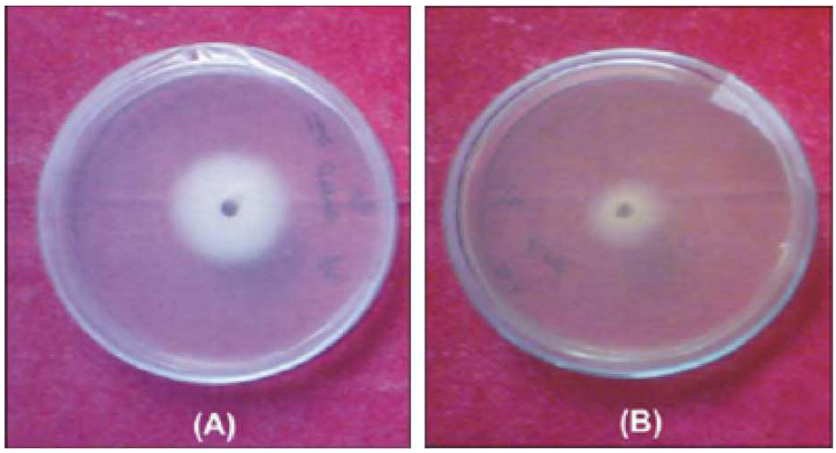

Figure 4
Anti-fungal activity of Aspergillus flavus of (A) Ligand (B) [Ni(L2)(L1)5]

Table 4
Antibacterial activities of the complexes - diameter of zone of inhibition (mm)

Compound	µg mL^{-1}	Gram Positive Bacteria		Gram Negative Bacteria		
		B.subtilis	*S.aureus*	*E.coli*	*P.*	*P. vulgaris*
Nicotinicacid hydrazide	30	09	09	06		07
	200	11	12	08	09	09
$[Cr(NHA)_3(SCN)_6]$	30	132	11	08	06	06
	200	156	18	07	07	08
$[Co(NHA)_2(SCN)_2]$	30	10	11	07	06	06
	200	11	13	06	06	07
$[Ni(NHA)_2(SCN)_2]$	30	12	10	06	06	06
	200	13	13	08	07	07
$[Cu(NHA)(SCN)_2]$	30	18	17	11	10	12
	200	22	23	13	14	14
$[Zn(NHA)(SCN)_2]$	30	13	12	08	07	07
	200	18	17	11	11	10
$[Hg(NHA)(SCN)_2]$	30	16	16	10	08	09
	200	19	21	14	12	12

5. DNA Binding

Electronic absorption study Electronic absorption spectroscopy was an effective method to examine the binding mode of DNA with metal complexes. In general, hypochromism and red-shift.

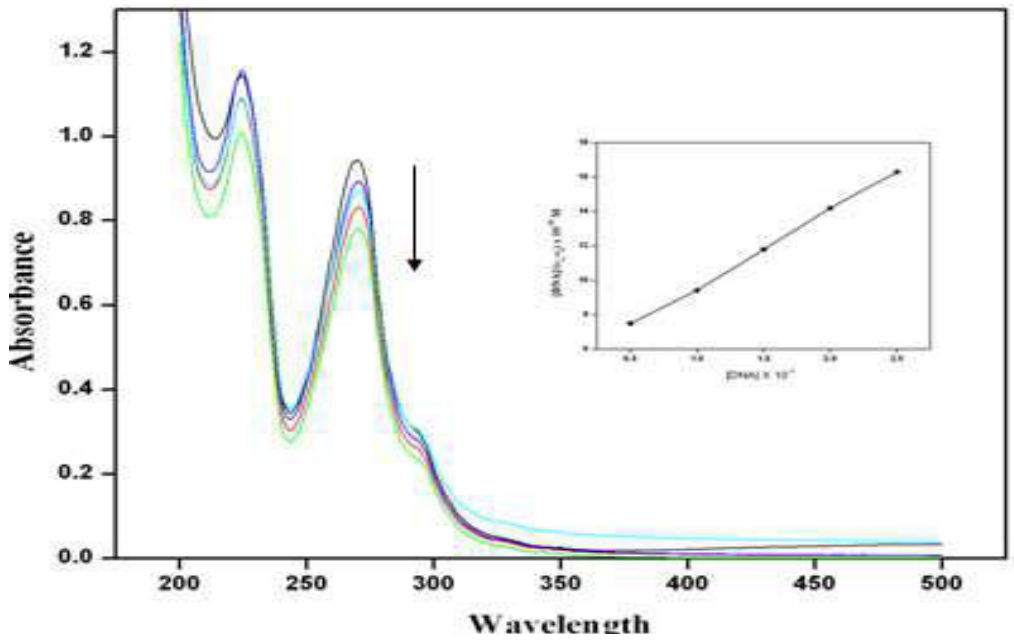

Figure 5
DNA Binding

Electronic Absorption spectra of [Cu(Phen)(L-Tyr)(TU)]ClO4. in the absence and in the presence of increasing amounts of DNA concentrations. [Complex] = 15 µM. [DNA] = (5,10,15,20,25) µM. Arrow shows the absorbance changes upon increasing DNA concentrations. are associated with the binding of the complex to the helix by an intercalative mode involving strong stacking interaction of the aromatic chromophore of the complex between the DNA base pairs. Fig. 2 shows the UV absorption spectra of copper(II) complex in the absence and presence of DNA. In the ultraviolet region from 240 to 300 nm, the complex had strong absorption peak at 265 nm, besides a shoulder band around 294 nm. The absorption intensity of the copper(II) complex sample decreased (hypochromism)

evidently after the addition of DNA, which indicated the interactions between DNA and the complex[11-12.] A minor red shift along with significant hypochromicity for the complex was observed. The copper(II) complex can bind to the double stranded DNA in different binding modes on the basis of their structure, charge and type of ligands. As DNA double helix possesses many hydrogen bonding sites which are accessible both in the minor and major grooves, it is likely that the –OH group of L-tyrosine ligand in the copper(II) complex form hydrogen bonds with DNA, which may contribute the hypochromism observed in the absorption spectra. The binding propensity of the phenanthroline complex is due to the presence of the extended planar aromatic ring in phenanthroline. The binding constant, Kb, was determined by using the following equation [DNA] / (εa-εf) = [DNA] / (εb-εf) + 1/ Kb (εb-εf) Where [DNA] is the concentration of DNA in base pairs, εa, εf and εb correspond to Aobsd/[Cu], the extinction coefficient of the free copper complex and the extinction coefficient of the complex in the fully bound form, respectively, and Kb is the intrinsic binding constant. The ratio of the slope to intercept in the plot of [DNA]/(εa - εf) versus [DNA] gives the value of Kb and for our copper(II) complex it is 4.52 X 10-5 M

6. ELECTROPHORESIS - DNA CLEAVAGE

The characterization of DNA recognition by transition metal complex has been aided by the DNA cleavage chemistry that is associated with redox-active or photo activated metal complexes. DNA cleavage is controlled by relaxation of super coiled circular form of pBR322 DNA into nicked circular form and linear form. When circular plasmid DNA is subjected to electrophoresis study, the fastest migration will be observed for the super coiled form (Form I). If one strand is cleaved, the super coils will relax to produce a slower-moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) will be generated which migrates in between. The DNA cleavage was analysed by monitoring the conversion of super coiled DNA (Form I) to nicked DNA (Form II) and linear DNA (Form III) in aerobic condition. Interestingly, we have found that this copper complex can cleave the super coiled DNA to nicked and linear DNA at the same time.

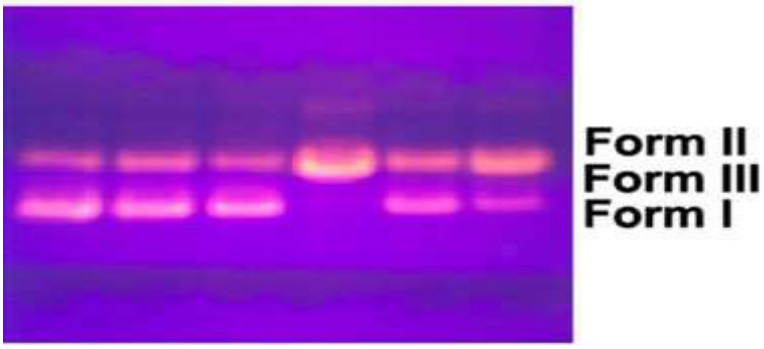

Figure 6

Electrophoretic behaviour of pBR322 DNA by [Cu(NHA)(SCN)$_2$]. Lane 1 pBR322 DNA alone: Lane 2-6: DNA + copper (II) complex in the concentration of 10, 15, 20, 30, 40 µM. As shown in Fig. 6, with the increase of the complex concentration, the intensity of the circular supercoiled DNA (Form I) band was found to decrease, while that of nicked

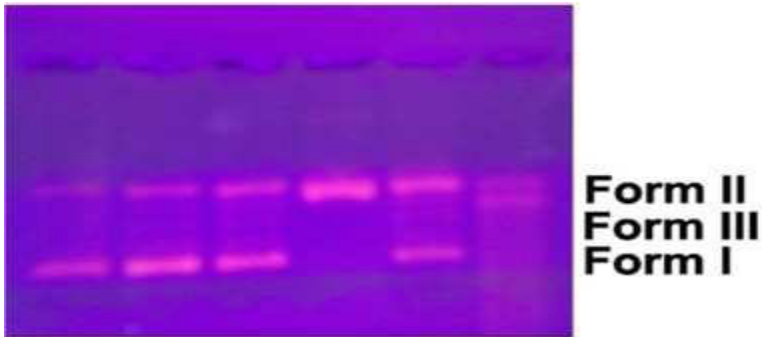

Figure 7

Electrophoretic separations of pBR322 DNA by [Cu(NHA)(SCN)$_2$] .Lane 1: DNA alone, Lane 2: DNA + Complex (25 µM), Lane 3: DNA + Ascorbic Acid (100 µM), Lane 4: DNA + Ascorbic Acid (100 µM) + Complex (25 µM), Lane 5: DNA + Ascorbic Acid (500 µM), Lane 6: DNA + Ascorbic Acid (500 µM) + Complex (25 µM). (Form II) and linear DNA (Form III) bands increase apparently.

When the complex concentration was up to 20 µM (lane 4), the circular supercoiled DNA (Form I) band disappeared completely. When it is more than 40 µM (lane 6), the circular supercoiled DNA (Form I) band becomes extremely faint. In order to establish the reactive species responsible for the cleavage of the plasmid DNA,we carried out the experiment in the presence of ascorbic acid as reducing agent (Fig. 7). Compared with the control experiments using only the copper (II) complex or ascorbic acid (lane 2, lane 3 and lane 5), the experiment using both copper (II) complex and the same concentration of ascorbic acid (lane 4 and lane 6) showed that the supercoiled DNA (Form I) apparently convert to nicked (Form II) and linear DNA (Form III). Although the ascorbic acid concentration in lane 5 was fivefold of that in lane 3, there is little difference between these two bands. When the same concentration of the copper (II) complex was added to them, an obvious difference occurred. Compared with lane 4, the supercoiled DNA (Form I) completely disappeared and the linear DNA (form III) apparently appeared in lane 6. These results are similar to that observed for some Cu-salen complexes as chemical nucleases. It is likely the generation of hydroxyl radical and/or activated oxygen mediated by the copper complex results in DNA cleavage. Further studies are being pursued to clarify the cleavage mechanism.

CONCLUSION

The present study deals with the preparation and characterization of transition metal complexes of 3-Pyridine carboxylic acid Hydrazide ion, five complexes were prepared with Cr(III), Co(II), Ni(II), Hg(II), Cd(II). These structures are assigned on the basis of analytical, conductance, magnetic measurement, UV, and IR spectral data. The super-coiled DNA is being cleaved in the electrophoresis by the complex which confirms that the complex is having the ability to act as a potent DNA cleavaging agent. The copper(II) complex exhibits good antimicrobial activity with their respective ligands.

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