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MICROWAVE SYNTHESIS, 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 NITRITE MIXED LIGAND COMPLEXES

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ABSTRACT

The complexes of Cr(III), Co(II), Ni(II), Hg(II), Cd(II) were microwave synthesized with the ligands Nicotinicacidhydrazide and $(ONO)_2$ and characterized by using Elemental analysis, Molar conductance measurements, IR, NMR, spectroscopic studies. DNA-binding properties have been studied by electronic absorption, emission, viscosity and cyclic voltametric methods. The results suggest that the copper (II) complex bind to DNA via different modes. Gel electrophoresis study reveals the fact that the copper complex cleaves super coiled pBR 322 DNA to nicked and linear forms in the presence and absence of nicotinicacidhydrazide. The in vitro antimicrobial study indicates that the complex has good activity against gram positive, gram negative bacteria and fungus. The fungal activity data shows that the metal complexes are potent active then the parent ligand NHA.

INTRODUCATION

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 (Sallam and Abbas, 2013). Pyridines derivative are very important in biological activities such as anti tubercular, anthelmintic, fungicidal, antitumor and antibacterial activities (Sidhaya *et al*., 2011; Rakesh Narang *et al*., 2012 and Ramalakshmi *et al*., 2009). NHA act as bidentate ligand sometimes it will act as monodentate ligand and having good ligating character, enhanced biological properties. The present study is aimed at the preparation and structural elucidation of a few nicotinicacidhydrazide (NHA) complexes by spectroscopic methods (UV and IR). In addition, a detailed analysis about the stereochemistry aspects of their metal complexes is obtained from spectral measurements. Comparisons of the IR Spectrum of nicotinic acid with those of the metal complexes were useful in determining the atoms of the ligand that are coordinated with the metal ion. Magnetic and electronic spectral measurements have been done for the coloured complexes.

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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 was measured using a Systronic conductivity bridge at room temperature in DMSO. Conductivity measurements (Ω^{-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 bacteria and fungus species were obtained from National Chemical Laboratory (NCL), Pune, India.

Electrochemical measurements were recorded on an Electrochemical analyser CH Instrument version 5.01 and model-600C. A three-electrode system comprising a glassy carbon working electrode, a platinum wire auxiliary electrode and a saturated calomel reference (SCE) electrode was used for voltametric work. 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.

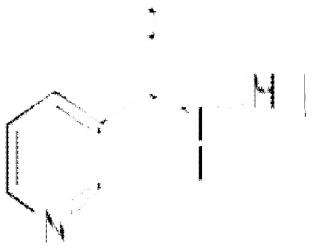

Fig. 1. Structure of NHA

(Fig.1). The direct constant parameters like A, B, C, q, p, y, nd v (volume) are given in Table 3 (Kazoo Nakamoto, 1978).[9]

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. 4.

Table 1. Microwave synthesis of metal complexes

S. No.	Weight of metal nitrate/ chloride in 10ml ethanol	Weight of NHA in 20 ml methanol	Condition	Colour of Solution/ precipitate	Weight of sodium nitrite in 10 ml ethanol	Condition	Colour of Complex
1	1.00g (3.40 mmol) $[Cr(NO_3)_2.9H_2O]$	0.71g (3.79 mmol)	Microwave irradiation for 10 seconds	Dark blue solution	0.49 g (7.53 mmol)	Microwave irradiation for 10 seconds	Grey precipitate
2	1.00g (3.40 mmol) $[Co(NO_3)_2.6H_2O]$	1.31g (6.93 mmol)		Pale pink precipitate	0.45 g (6.90 mmol)		Pink precipitate
3	1.00g (3.40 mmol) $[Ni(NO_3)_2.6H_2O]$	1.31g (6.93 mmol)		Sky blue precipitate	0.45 g (6.90 mmol)		Pale blue precipitate
4	1.00g (4.20 mmol) $[Cu(NO_3)_2.3H_2O]$	0.79g (4.14 mmol)		Greenish blue precipitate	0.54g (8.40 mmol)		Green precipitate
5	1.00g (3.33 mmol) $[Zn(NO_3)_2.6H_2O]$	0.63g (3.36 mmol)		Colourless precipitate	0.43g (6.62 mmol)		Colourless precipitate
6	1.00g (3.22 mmol) $[Cd(NO_3)_2.4H_2O]$	0.61g (3.21 mmol)		Colourless precipitate	0.42g (6.46 mmol)		Colourless precipitate
7	1.00g (3.64 mmol) $HgCl_2$	0.69g (3.64 mmol)		Colourless precipitate	0.47g (7.23 mmol)		Colourless precipitate

Table 2. Analytical data and molar conductance of the complexes

S. No.	Complex	% H	% N	% C	% M	Yield (%)	Λm ($\Omega^{-1}cm^2mol^{-1}$)
1	$[Cr_2(ONO)_6(NHA)_2]$	2.65 (2.60)	26.53 (26.51)	27.29 (27.24)	13.14 (13.17)	48.00	95.3
2	$[Co(ONO)_2(NHA)_2]$	3.29 (3.28)	26.33 (26.31)	33.86 (33.85)	13.85 (13.83)	66.83	98.5
3	$[Ni(ONO)_2(NHA)_2]$	3.11 (3.10)	24.94 (24.93)	37.41 (37.40)	13.07 (13.05)	75.70	94.4
4	$[Cu(ONO)_2(NHA)]$	2.39 (2.37)	23.91 (23.89)	24.59 (24.57)	21.71 (21.70)	81.21	69.9
5	$[Zn(ONO)_2(NHA)]$	2.37 (2.35)	23.76 (23.74)	24.44 (24.40)	22.20 (22.18)	64.57	80.5
6	$[Cd(ONO)_2(NHA)]$	2.04 (2.02)	20.49 (20.47)	21.08 (21.05)	32.91 (32.90)	76.66	79.0
7	$[Hg(ONO)_2(NHA)]$	1.62 (1.63)	16.28 (16.26)	16.75 (16.73)	46.67 (46.63)	77.67	99.8

Table 3. IR Spectral data of NHA and its metal complexes (Cm^{-1})

S. No.	Complex	ν_1 (nm)	ν_2 (nm)	ν_3 (nm)
1	$[Cr_2(ONO)_6(NHA)_2]$	563	420	264
2	$[Co(ONO)_2(NHA)_2]$	522	448	320
3	$[Ni(ONO)_2(NHA)_2]$	583	294	210
4	$[Cu(ONO)_2(NHA)]$	686	518	Obscured by C-T
5	$[Zn(ONO)_2(NHA)]$		269	
6	$[Cd(ONO)_2(NHA)]$		277	
7	$[Hg(ONO)_2(NHA)]$		266	

RESULTS AND DISCUSSION

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

Comparative studies of the ligands and their complexes indicated that metal complexes exhibit higher antifungal activity than the free ligands. 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 com- pared to the standard drug clotrimazole.

Table 4. X-ray powder pattern reports Powder x-ray diffraction data for $[Cr_2(ONO)_6(NHA)_2]$

Complex	2θ values	Unit Cell parameters	Density (g/cc)	Possible geometry
$[Cr_2(ONO)_6(NHA)_2]$	14.564	a = 7.0696 Å,	2.1824	Monoclinic
	15.216	b = 14.8954Å,		
	17.421	c = 5.3504 Å		
	18.508	α =90°		
	19.410	β=108°		
	19.544	γ =90° V=563Å^3		
	20.580			
	23.036			
	23.939			
	26.290			
	26.796			
	32.194			
	33.331			
	40.533			
	42.939			
	43.524			
	45.129			
	47.669			
	48.588			
	49.474			
	51.161			
	51.312			
	52.699			
	54.454			
	54.821			
	55.256			
	55.356			
	55.557			
	56.960			
	57.428			
	57.545			
	57.746			
	58.046			
	58.113			
	58.264			
	58.464			
	59.116			
	59.367			
	59.901			

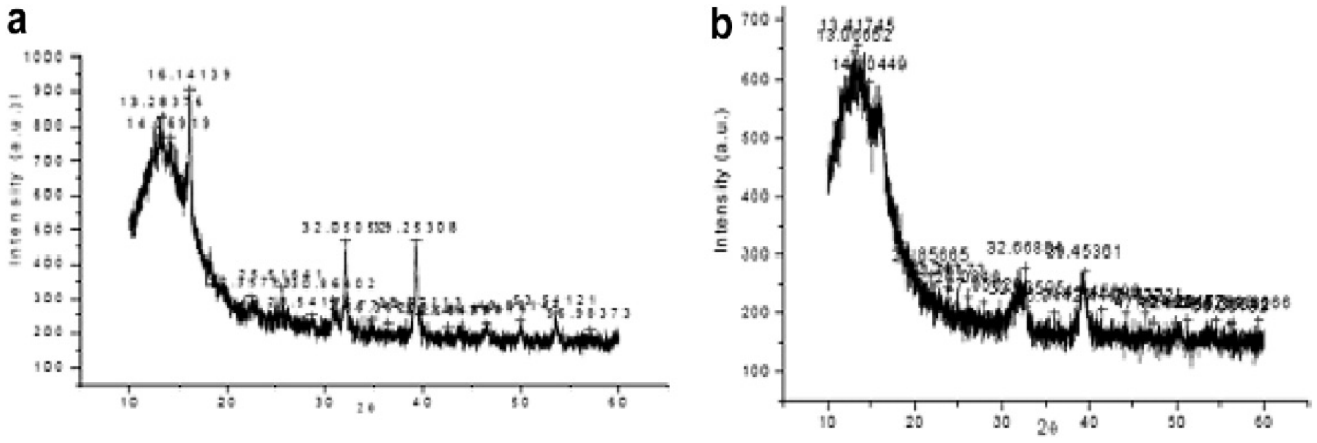

Fig. 2. XRD (Powder Pattern) of the complexes (a) $[Cr_2(ONO)_6(NHA)_2]$ and (b) $[Ni(ONO)_2(NHA)_2]$

The order of the me- tal complexes follow 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 overtons 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.

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 260 nm. 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. 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 (η/η0)1/3 versus binding ratio, where η is the viscosity of CT DNA in the presence of complex, and η0 is the viscosity of CT DNA alone.

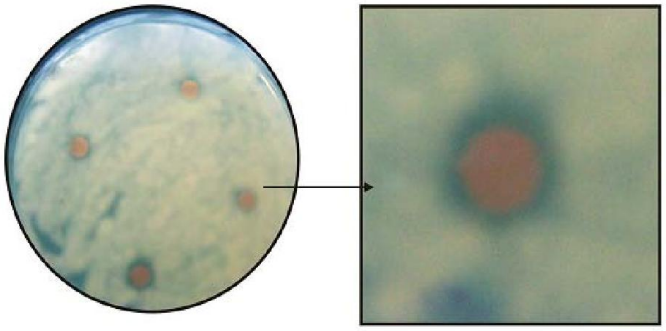

Fig. 4. Antibacterial activity of the chromium complex against the *P.aeruginosa*

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

DNA Binding Studies

3.1 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 Fig.5. 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.

We have observed minor red shift along with significant hypochromicity for the complex. 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

Electrophoresis - DNA Cleavage

The characterisation 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. 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.

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. their respective ligands.

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