

Synthesis, Characterisation and Biological Evaluation of Nalidixic Acid Metal Complexes

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ABSTRACT

Metal complexes of Nalidixic acid such as $[\text{Cu}(\text{NDX})_2] \cdot 2\text{H}_2\text{O}$, $[\text{Zn}(\text{NDX})_2] \cdot 2\text{H}_2\text{O}$, $[\text{Ni}(\text{NDX})_2] \cdot 6\text{H}_2\text{O}$, and $[\text{Fe}(\text{NDX})_2(\text{H}_2\text{O})\text{Cl}] \cdot 5\text{H}_2\text{O}$ have been synthesized and characterised by elemental analysis, IR, UV-Vis, ESR, NMR, XRD spectroscopic techniques. The results of elemental analysis show that two molecules of the tioconazole drug are attached per metal ion. Thermal analysis was studied by TGA, DSC method. Morphological studies were carried out by scanning electron microscopic method. Further the complexes were screened for their antimicrobial property. The results of elemental analysis show that two molecules of the tioconazole drug are attached per metal ion. From the spectral studies a square planar geometry was proposed for the copper complex. Tetrahedral geometry for zinc and nickel complex and octahedral geometry for iron complex. In conclusion, prepared complexes showed enhanced biological activities than the parent drug that might be of interest for future research.

Keywords: Antimicrobial, geometry, ligand, metal complexes, nalidixic acid.

1. INTRODUCTION

Study of the interaction between drugs and metal ions is an important and active research area in bioinorganic chemistry [1-4]. It is well known that the action of many drugs is dependent on the coordination with metal ions or the inhibition on the formation of metalloenzymes. Quinolones considered a class of antibacterial agents which have been known for over 40 years. Presence of metal ions considerably alters the activity of quinolones against potentially susceptible bacteria. The interaction of metal ions with diverse deprotonated quinolones as ligands has been thoroughly discussed [5]. Quinolones have been classified in generations based on their activity [6]. Each generation presents an enhanced spectrum of activity in comparison to a previous one. Nalidixic acid is an example of first-generation quinolones that are active against Gram-negative organisms and they are used for the treatment of uncomplicated urinary tract infections [7]. The association of complexes resulted between nalidixic acid and different metal ions has been described [8]. Therefore, the importance of this paper is to study the interaction between the transition metal ions with nalidixic antibiotic ligand.

2. EXPERIMENTAL DETAILS

Complexes with the general formula $[\text{M}(\text{NDX})_2] \cdot n\text{H}_2\text{O}$ have been synthesized, employing a 1:2 (metal ions:NDX) ratio. A solution of 0.1M of a salt of each Zn (II), Cu (II), Ni(II) and Fe(III) previously dissolved in 10 ml of distilled water was added to a solution of 0.1 M of norfloxacin in 20 ml of acetone. The resulting mixtures were heated to 50 °C under reflux on a water bath for about 2 hours and then cooled. The complexes obtained were separated from the reaction mixture by filtration, washed with boiling water and acetone and dried under vacuum over CaCl_2 . The complexes are formulated as $[\text{Cu}(\text{NDX})_2] \cdot 2\text{H}_2\text{O}$, $[\text{Zn}(\text{NDX})_2] \cdot 2\text{H}_2\text{O}$, $[\text{Ni}(\text{NDX})_2] \cdot 6\text{H}_2\text{O}$, and $[\text{Fe}(\text{NDX})_2(\text{H}_2\text{O})\text{Cl}] \cdot 5\text{H}_2\text{O}$.

3.3 Electronic spectral studies

The electronic spectrum of the $[\text{Cu}(\text{NDX})_2] \cdot 2\text{H}_2\text{O}$ complex (Fig 2) shows a broad absorption band between 600–700 nm which is assigned to ${}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{1g}$ transition, indicating a square planar geometry of Cu(II) complex [10]. The square planar geometry of Cu(II) in the complexes is confirmed by the measured magnetic moment values of 1.73–1.81 B.M. The Zn(II) complex does not exhibit d–d electronic transition due to completely filled d orbital. Four coordinate Zn(II) complexes, in general, would have tetrahedral geometry. The electronic spectrum of Ni(II) complexes shows only one band in the visible region at 620 nm which is assigned to ${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{P})$ transition for tetrahedral geometry. From the spectral data (Table 3) of the iron(III) complexes it can be seen that all of them exhibit one band at 508–568 nm which can be assigned to ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{1g}$ transition characteristic of octahedral structure. The broad intense and poorly resolved bands between 320–450 nm may be assigned to MLCT [11]. The high intensity band below 320 nm is of ligand origin assignable to intraligand $n - \pi / \pi - \pi^*$ transition [12].

Table 3. Electronic spectral data of $[\text{Cu}(\text{NDX})_2] \cdot 2\text{H}_2\text{O}$

Compound	Transition	Wavelength	Geometry	μ_{eff}
Nalidixic acid	$n - \pi / \pi - \pi^*$	below 250	-	-
$[\text{Cu}(\text{NDX})_2] \cdot 2\text{H}_2\text{O}$	${}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{1g}$	600-700	square planar	1.73-1.81
$[\text{Zn}(\text{NDX})_2] \cdot 2\text{H}_2\text{O}$	-	-	Tetrahedral	diamagnetic
$[\text{Ni}(\text{NDX})_2 \cdot 6\text{H}_2\text{O}]$	${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{P})$	620	tetrahedral	3.26
$[\text{Fe}(\text{NDX})_2(\text{H}_2\text{O})\text{Cl}] \cdot 5\text{H}_2\text{O}$	$\text{A}_{2g}(\text{F}) \rightarrow \text{T}_{2g}(\text{F})$ $\text{A}_{2g}(\text{F}) \rightarrow \text{T}_{1g}(\text{F})$ $\text{A}_{2g}(\text{F}) \rightarrow \text{T}_{1g}(\text{P})$	983, 755, and 506	octahedral	5.04

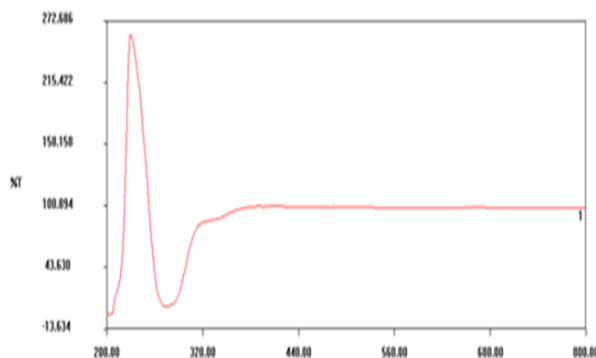


Fig 2. Electronic spectrum of $[\text{Cu}(\text{NDX})_2] \cdot 2\text{H}_2\text{O}$

3.4 ${}^1\text{H}$ NMR spectrum

The ${}^1\text{H}$ NMR spectrum further supports the assignment of the coordination modes. In the free ligand, the signal observed at 13.34 ppm can be assigned to the carboxylate –OH group. This signal disappears in the spectrum of the $[\text{Zn}(\text{NDX})_2] \cdot 2\text{H}_2\text{O}$ complex, which confirms the coordination of ligand to the M(II) ions through the deprotonated carboxylic –OH group. The peak at δ 3.55 ppm can be assigned as coming from the water molecules of crystallization, which were not detected in the spectrum of the free ligand. The protons of the – CH_3 – group of pyridine appears at δ 2.712 ppm, while the protons – CH_2CH_3 group (triplet) at δ 4.630 ppm. From the spectral data it is concluded that the ligand coordinate with the metal ions through pyridone and one carboxylic oxygen atom.

3.5 ESR spectrum

ESR spectrum of the $[\text{Cu}(\text{NDX})_2] \cdot 2\text{H}_2\text{O}$ complex (Fig 3) was recorded in DMSO at 300 K and 77 K. The magnetic susceptibility value reveals that Cu(II) complex has a magnetic moment of 1.86 BM indicating the presence of one unpaired electron, showing that the complex is mono nuclear in nature. This fact is also evident from the absence of a half filled signal observed in the ESR spectrum at 1600 G due to the $\Delta m_s = \pm 2$ transition, ruling out any Cu–Cu interaction. In the cefixime–Cu complex, the observed trend of $g_{\parallel} (2.354) > g_{\perp} (2.256) > g_e (2.0036)$ indicated that the unpaired electron is localised in the $d_{x^2-y^2}$ orbital of the Cu (II) ion [13]. The A_{\parallel} and

A_{\perp} values in the order A_{\parallel} ($150 > A_{\perp}(36.5)$) also indicate that the complex has a square planar geometry and the system is axially symmetric [14]. Molecular orbital coefficients α^2 (in plane σ bonding), β^2 (in plane π bonding) and γ^2 (out-plane π bonding) were calculated using equation [15]. In the present study, the observed γ^2 value is 0.7125 which indicates the complex has some covalent character in the ligand environment. The observed α^2 and β^2 values of 1.256 and 0.7321 indicate that there is an interaction in the out of plane π bonding (Table 4). The observed values of $k_{\parallel}(0.82) > K_{\perp}(0.533)$ imply a greater contribution from the out of plane π bonding in metal–ligand π bonding.

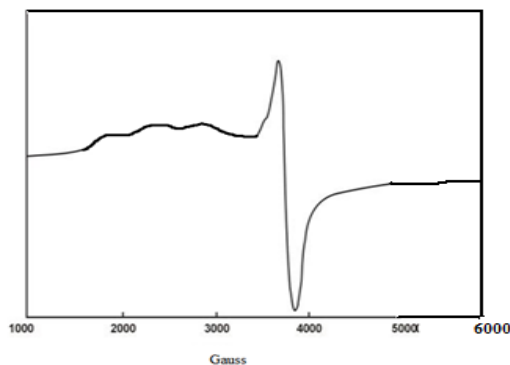


Figure 3 ESR spectrum of $[\text{Cu}(\text{NDX})_2] \cdot 2\text{H}_2\text{O}$

Table 4 ESR spectral data of $[\text{Cu}(\text{NDX})_2] \cdot 2\text{H}_2\text{O}$

Complex	\parallel	\perp	iso	\parallel	\perp	2	2	2	
$[\text{Cu}(\text{NDX})_2] \cdot 2\text{H}_2\text{O}$ 77K	.315	.087		.772	.664	.754	.716	.715	.2
$[\text{Cu}(\text{NDX})_2] \cdot 2\text{H}_2\text{O}$ 300K	-	-	.10						

3.6 XRD studies

The XRD pattern of metal complexes complex (Fig 4) was studied in the 2θ range of $5-35^\circ$. The crystalline size of the complex was calculated from Scherer's formula. From the observed d_{XRD} pattern, the average crystalline sizes of the metallo drugs indicates that they are nanocrystalline in nature.

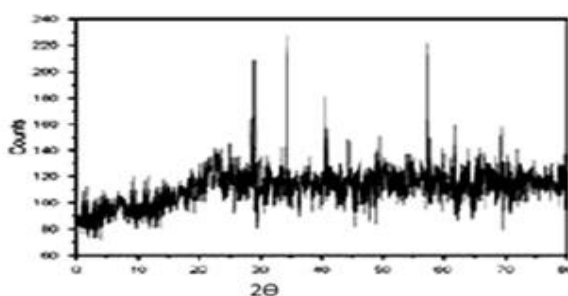


Fig 4 Powder XRD pattern of $[\text{Cu}(\text{NDX})_2] \cdot 2\text{H}_2\text{O}$

3.7 TGA studies

The TGA studies were carried out to explore the thermal stability of the complexes. The thermal behaviour of the metal complex (Fig 5) was studied in the temperature range of $25-800^\circ\text{C}$. The TGA studies of complex $[\text{Cu}(\text{NDX})_2] \cdot 2\text{H}_2\text{O}$ reveal that the decomposition proceeds in three steps. In the first stage weight loss at 118°C corresponds to the presence of the lattice water. The weight loss in the temperature range $330-350^\circ\text{C}$ is due to the decomposition of organic moiety. A plateau was obtained after heating above 400°C , which corresponds to the formation of stable metal oxide [16,17]. The TGA studies of complex $[\text{Fe}(\text{NDX})_2(\text{H}_2\text{O})\text{Cl}] \cdot 5\text{H}_2\text{O}$ reveal that the decomposition proceeds in four steps. In the first stage weight loss at 118°C corresponds to the presence of the lattice water. The weight loss in the temperature range $220-230^\circ\text{C}$ is due to the presence of the coordinated water. The weight loss in the temperature range $260-280^\circ\text{C}$ is due to the presence of chlorine molecule. The weight loss in the temperature range $360-380^\circ\text{C}$ is due to the decomposition of organic moiety. A plateau was obtained after heating above 500°C , which corresponds to the formation of stable metal oxide.

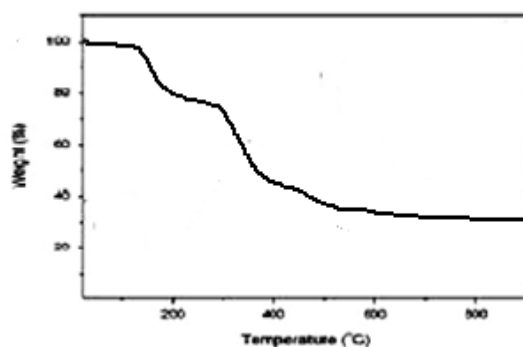


Fig 5 TGA pattern of [Cu(NDX)₂]. 2H₂O

3.8 SEM studies

Layers in the micrograph of the complexes reveal that the system (Fig6) contains atoms in a well-defined pattern. Thus reactants have reacted completely to form a clear homogenous compound. In general the SEM photograph shows single phase formation with well-defined grain like shape and particle size in the range of 0.5 μm .

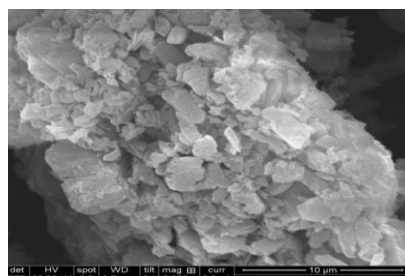


Fig 6 SEM image of [Cu(NDX)₂]. 2H₂O

3.9 FAB mass spectrum

The FAB mass spectrum of the Cu(II) complex (Fig 7) gives a molecular ion peak M^+ at m/z 232 which corresponds to the molecular mass of the ligand. The observed molecular ion peaks at m/z 461 accounts for the molecular mass of the complex [Cu(NDX)₂]. 2H₂O. The other peaks observed at m/z 29 and m/z 158 corresponds to the molecular weight of the fragments $C_2H_5^+$ and $C_9H_6N_2O$ respectively.

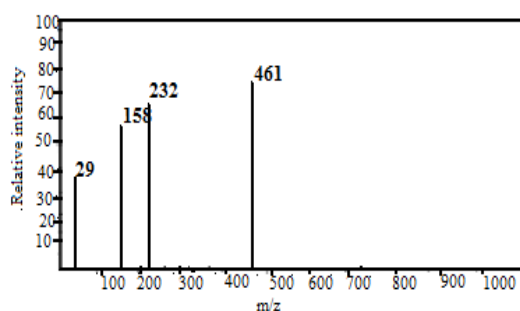


Fig 7 FAB mass spectrum of [Cu(NDX)₂]. 2H₂O

4. Biological studies

4.1. Antimicrobial studies

Antimicrobial resistance is fast becoming a global concern with rapid increase in multidrug-resistant bacteria. Some previously treatable pathogens are now becoming untreatable, for example methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *enterococcus* (VRE) [18]. Some other organisms are also showing drug resistance like *M.tuberculosis* (Strain no: H₃₇Rv ATCC 27294), susceptible to rifampicin, isoniazid, streptomycin, and ethambutol and two other clinical strains of multidrug-resistant *M.tuberculosis* (MDRTB) not susceptible to isoniazid and rifampicin and *Klebsiella pneumonia* is another drug resistant bacteria. This also extends to other gram negative organisms like *Escherichia coli*, *Shigella flexneri*, *Pseudomonas aeruginosa*, and *Salmonella typhi* and Gram-positive *Bacillus subtilis* bacterial strains. Even some

fungus pathogens are also showing the resistance feature against drugs like *Candida albicans*, *Aspergillus flavus*, *Fusarium solani*, and *Candida glabrata*.

4.2 Results and discussion

Antibacterial activity of the ligands/drugs and their complexes were tested against the bacterial species *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Pseudomonas aeruginosa* by Kirby Bauer Disc diffusion method [19]. Each experiment was carried out in triplicate, and the microbial activity was determined by measuring the diameter of zone of inhibition in mm. The MIC values were determined by serial dilution technique [20] against bacterial species. The metal complexes showed enhanced activity against parent drugs. Such increased activity of the complexes can be explained on the basis of Overtone's concept and Tweedy's Chelation Theory [21]. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favours the passage of only the lipid soluble materials due to which liposolubility is an important factor, which controls the antifungal 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 charge of the metal ion with donor groups. Further, it increases the delocalization of π -electrons over the whole chelate ring and enhances the lipophilicity of the complexes. This increased lipophilicity enhances the penetration of the complexes into lipid membrane and restricts further multiplicity of the microorganisms.

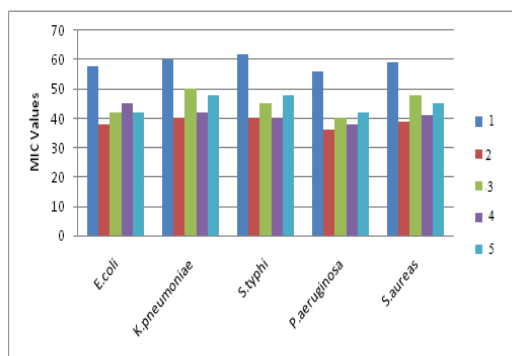


Fig 8 Minimum inhibitory concentrations of Nalidixic acid and its metal complexes against bacterial species ($\mu\text{g/ml}$)

1. Nalidixic acid 2. $[\text{Cu}(\text{NDX})_2] \cdot 2\text{H}_2\text{O}$, 3. $[\text{Zn}(\text{NDX})_2] \cdot 2\text{H}_2\text{O}$, 4. $[\text{Ni}(\text{NDX})_2] \cdot 6\text{H}_2\text{O}$
 5. $[\text{Fe}(\text{NDX})_2(\text{H}_2\text{O})\text{Cl}] \cdot 5\text{H}_2\text{O}$.

CONCLUSIONS

The study of the reaction between the metal complexes and the drug indicates its high stability. This encourages the synthesis and careful investigation of the nature of bonding between the drug and the transition metal ion of important biological role, using physicochemical method of analysis. It is clear from above discussion that the fragmentation pattern and spectral studies of the complex confirm and illustrate the proposed geometry obtained by elemental analysis, IR, ^1H NMR, and mass spectra.

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