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ORIGINAL ARTICLE

Designing of some novel metallo antibiotics tuning biochemical behaviour towards therapeutics: Synthesis, characterisation and pharmacological studies of metal complexes of cefixime

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Abstract Cefixime is a broad spectrum semi synthetic cephalosporin antibiotic for oral administration. Metal complexes of cefixime with Cu(II), Zn(II), Cd(II), Fe(III) and Ni(II) have been synthesised and characterised by elemental analysis and IR, UV–Vis., NMR and ESR spectra. The electronic spectral behaviour and cyclic voltammetric studies have been carried out on the interaction of metal complexes with calf thymus DNA. The results suggest that the complexes can bind to DNA by intercalation mode. The Cu(II), Zn(II), Cd (II) and Ni(II) complexes exhibit square planar geometry. Fe(III) complex exhibits octahedral geometry. The complexes showed a slightly higher antimicrobial activity than the cefixime drug. Among the metal complexes, Fe(III) was found to be more active than other complexes when tested against bacterial species *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* and fungal species *Aspergillus niger*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Rhizoctonia bataicola* and *Candida albicans* by the disc diffusion method. SEM analysis provides the morphology of the metal complexes. The DNA binding interaction of metal complexes with CT DNA using the cyclic voltammetry technique and their salient features are discussed.

1. Introduction

Over the past few decades, intensive research efforts have been made to design novel compounds to deal with new strains of resistant micro-organisms. The on-going literature search for innovative drug delivery systems is predominantly a consequence of the well-established fact that the conventional dosages are not sufficiently effective in conveying the drug compounds to its site of action and this has necessitated the need to search for more potent drugs. The recognition of the

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chelates in therapeutic application provides useful outlets for outstanding research in transition metal chemistry.

Antibiotics can interact with a variety of biomolecules which may result in the inhibition of the biochemical or biophysical processes associated with biomolecules (David et al., 1992). There are a number of antibiotics that require metal ions to function properly such as bleomycin (BCM), Streptonigrin (sn) and Bacitracin. Metallo-antibiotics can interact with several biomolecules such as DNA, RNA, protein receptors and lipids, making them very unique and specifically bioactive. The coordinated metal ions in these antibiotics play an important role in determining proper structure and function of these antibiotics (Li-june, 2003). Cefixime is a semi synthetic third generation cephalosporin antibiotic for oral administration. Chemically it is 7-2(2-(amino-4-thiazolyl)-2(carboxymethoxyimino)acetamido)-3-vinyl-cephem-4-carboxylic acid (Ali, 2002), having molecular weight 507.50 as the trihydrate. The metal complexes play an essential role in pharmaceutical industry and in agriculture. The metallo-elements present in trace quantities play a vital role at the molecular level in the living system. The transition metal ions are responsible for proper functioning of different enzymes. The activity of bio metals is attained through the formation of complexes with different bioligands and the mode of biological action of complexes depends upon the thermodynamic and kinetic properties. The lipophilicity of the drug is increased through the formation of chelates and drug action is significantly increased due to effective permeability of the drug into the site of action. Interaction of various metal ions with the antibiotics may enhance their antimicrobial activity as compared to that of free ligands (Hariprasath et al., 2010).

Many drugs in the form of metal complexes possess modified toxicological and pharmaceutical properties .The most widely studied metal is copper (II) which has proved beneficial in diseases such as tuberculosis, gastric ulcers, rheumatoid arthritis and cancers (metals of life, 1971). These results encouraged us to investigate the coordination chemistry of antibiotics with transition and d^{10} metal ions in an attempt to examine the modes of binding in solid state and to study the biological activity.

Keeping in view of the importance of drug molecules, the present research work is focused on the synthesis and characterisation of metalloantibiotics (metal–cefixime). Further, to evaluate the changes in the antimicrobial activity of cefixime after complex formation with Cu(II), Zn(II), Cd(II), Ni(II) and Fe(III) against bacterial species *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa* and fungal species *Aspergillus niger, Rhizopus stolonifer, Aspergillus flavus, Rhizoctonia bataicola* and *Candida albicans* by the disc diffusion method.

2. Materials and methods

All chemicals were of reagent grade purchased from Sigma and used without further purification. Solvents were redistilled by a standard technique before use.

2.1. Physical methods

C, H, N and S were analysed in a ELECO CHNS 932 model micro analytical instrument. The metal content of each complex was determined by atomic absorption spectroscopy. The IR spectra of the ligand and metal complexes were recorded in KBr pellets in the 4000–400 cm^{-1} range with a Perkins Elmer series 2000 spectrophotometer. UV–Visible spectra were recorded using a Perkin–Elmer recording spectrometer. Electronic absorption spectra were recorded in DMSO using the UV–Vis., double beam spectrometer 2201. Molar conductance of the copper complexes was measured in DMSO solution using a coronation digital conductivity meter. The magnetic susceptibility values were calculated using the relation $\mu_{eff} = 2.83(\chi_m .T)$. The diamagnetic corrections were made by Pascal's constant and $Hg[Co(SCN)_4]$ was used as a calibrant. Electrochemical experiments were performed on a CHI 604D electrochemical analysis system with a three-electrode system consisting of a glassy carbon working electrode, Pt wire auxiliary electrode and an Ag/AgCl reference electrode. Tetrabutylammoniumperchlorate (TBAP) was used as the supporting electrolyte. All solutions were purged with N_2 for 30 min prior to each set of experiments. EPR Spectrometer in DMSO solution was used both at room temperature (300 K) and at liquid nitrogen temperature (77 K) using TCNE (tetracyanoethylene) as the g marker.

2.2. DNA-binding assay

The interaction between metal complexes and DNA was studied using electrochemical methods. The disodium salt of calf thymus DNA was stored at 4 °C. A solution of DNA in the buffer containing 50 mM NaCl and 5 mM Tris HCl (pH 7.2) in water in a ratio of 1.9 gave UV absorbance at 260 and 280 nm, A_{260}/A_{280}, indicating that the DNA was sufficiently free from protein. The concentration of DNA was measured using its extinction coefficient at 260 nm (6600 M^{-1}) after 1:100 dilutions. Stock solutions were stored at 4 °C and used within 4 days. Doubly distilled water was used to prepare solutions. Concentrated stock solutions of the complexes were prepared by dissolving the complexes in DMSO and diluting suitably with the corresponding buffer to the required concentration for all the experiments.

2.3. Antimicrobial activity

The *in vitro* evaluation of antimicrobial activity was carried out. The prepared compounds were tested against some fungi and bacteria to provide the minimum inhibitory concentration (MIC) for each compound. MIC is the lowest concentration of solution to inhibit the growth of a test organism. The in vitro biological screening effects of the investigated compounds were tested against the bacterial species *S. aureus, E. coli, K. pneumoniae, P. vulgaris* and *P.* aeruginosa and fungal species *A. niger, R. stolonifer, A. flavus, R. bataicola* and *C.* albicans by the disc diffusion method. One day prior to the experiment, the bacterial and fungal cultures were inoculated in nutrient broth (inoculation medium) and incubated overnight at 37 °C. Inoculation medium containing 24 h grown culture was added aseptically to the nutrient medium and mixed thoroughly to get a uniform distribution. This solution was poured (25 ml in each dish) into petri dishes and then allowed to attain room temperature. Wells (6 mm in diameter) were cut in the agar plates using proper sterile tubes. Then, the wells were filled up to the surface of agar with 0.1 ml of the test compounds dissolved in DMSO (200 μg/ml). The plates were al-

lowed to stand for an hour in order to facilitate the diffusion of the drug solution. Then the plates were incubated at 37 °C for 24 h for bacteria and 48 h for fungi and the diameter of the inhibition zones was measured. Minimum inhibitory concentrations (MICs) were detected by the serial dilution method.

2.4. Synthesis of metal complexes

Equimolar ethanolic solutions of cefixime drug and metal salt ($FeCl_3.3H_2O$, $CuCl_2.6H_2O$, $NiCl_2.6H_2O$, $CdCl_2.6H_2O$) were mixed at room temperature. Then, the pH of the solution mixture was adjusted to 8.0 using 0.5 M NaOH. The precipitated complexes were filtered off, washed with ethyl alcohol and dried in vacuo. The molar conductance values of the metal complexes (measured in 10^{-3} M DMSO) are in the range of 3–6 $\Omega^{-1}\,cm^2\,mol^{-1}$ indicating the non electrolytic nature. The purity of metal complexes has been checked by TLC.

3. Results and discussion

Cefixime has two ionisable carboxyl groups and so exists predominantly as a dianion. The elemental analysis was in good agreement with a 1:1 metal to drug stoichiometry for all the complexes (Table 1). Thermogravimetric analysis of the hydrated complexes indicated that the endothermic decomposition in the 155–172 °C range is due to the loss of water molecules from the coordination sphere. The general molecular formula [Fe(cefixime)(H_2O)Cl] and [M(cefixime)(H_2O)$_2$] have been assigned to the complexes based on the analytical and spectral data.

Table 1 Elemental analysis and molar conductance values of compounds.

Compounds	Found (calcd) %			
	C	H	N	M
Fe(cef)(H_2O)CL	34.6(34.3)	2.7(2.8)	11.9(11.8)	11.7911.4)
Zn(cef)(H_2O)$_2$	31.5(31.2)	2.7(2.5)	12.8(12.2)	11.5(11.2)
Cu(cef)(H_2O)$_2$	32.5(32.4)	2.9(2.8)	11.8(11.7)	12.1(12.2)
Cd(cef)(H_2O)$_2$	31.7(32.1)	3.1(3.0)	11.4(11.7)	11.5(11.1)
Ni(cef)(H_2O)$_2$	35.4(35.2)	2.9(3.1)	12.9(12.8)	12.1(11.7)

3.1. IR spectra

The IR spectra of the free drug were compared with those of the metal complexes in order to ascertain the bonding mode of the drug to metal ion in the complexes. The lactam band appears at 1766 cm^{-1} in the free cefixime while in the copper complex (Fig. 1) appears at 1766 cm^{-1} suggesting that no coordination occurs with copper ion. The N–H stretching frequency of amide carbonyl band in the free cefixime appears at 1674 cm^{-1} with a weak shoulder at 1635 cm^{-1} while in the complex shows this band at 1676 cm^{-1} with a prominent peak at 1631 cm^{-1} indicating the coordination of cefixime with Cu(II) through nitrogen atom of amide carbonyl group. The asymmetrical and symmetrical stretching bands of carboxylate groups were observed in the range from 1533–1543 cm^{-1} and 1373–1379 cm^{-1} respectively due to coordination .The M–N stretching vibration occurs at 428 cm^{-1} (Ghazy El-Shazly and El-S, 2006). In the spectra of metal complexes a broad band in the region 3300–3420 cm^{-1} indicated the presence of coordinated water molecules. In the case of [Fe(cefixime)(H_2O)Cl], the appearance of a new band at 390 cm^{-1} is assigned to M–Cl band. From the IR spectral features it is clear that the drug molecule is bonding through amide nitrogen atom, carboxyl oxygen atom of thiazole moiety and oxygen atom of water molecules, respectively.

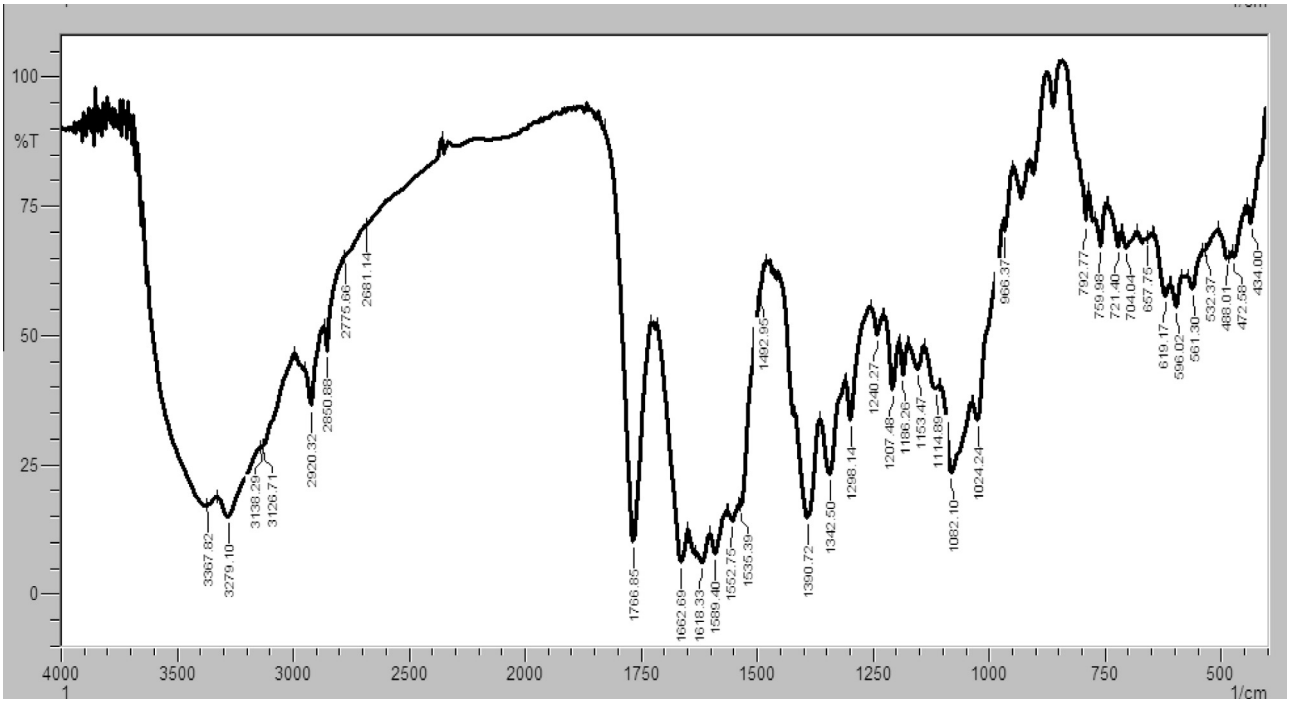

Figure 1 IR spectrum of copper complex.

3.2. Electronic spectra

The electronic spectrum of Cu–cefixime complex shows a broad absorption band between 600–700 nm which is assigned to $B_1g \rightarrow A_1g$ transition. This indicates the square planar geometry of Cu(II) complex Lever, 1984. The square planar geometry of Cu(II) in the complexes is confirmed by the measured magnetic moment values, 1.73–1.81 BM.

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 Cd(II) complexes shows only one band in the visible region at 620 nm which is assigned to $A_2(F) \rightarrow T_1(P)$ transition for tetrahedral geometry. The electronic spectra of Fe(III) complex shows three bands at 983, 755, and 506 nm which are assigned to $A_2g(F) \rightarrow T_2g(F)$, $A2\ g \rightarrow T1\ g(F)$ and $A_2g(F) \rightarrow T1\ g(p)$ transitions, respectively. The results were in accordance with octahedral geometry for Fe(III)–cefixime complex (Jergensen, 1972).

3.3. NMR studies

The ^1H NMR spectrum of Zinc complex in $CDCl_3$ solution shows the following signals: amine proton at 7.2 ppm (2H, s), S-*CH* at 7.5 ppm (1H, s), propiolactam proton appears at 5.2 ppm (3H, t), ethylene proton ($CH{\equiv}CH_2$) at 4.5 ppm as a triplet and $CH{\equiv}CH_2$ at 6.5 ppm, carboxylic methylene proton at 1.4 ppm (2H, s), amide proton (CO–*NH*) at 8.3 ppm, carboxylic protons of lactum and thiazole moieties at 11.4 and 12.3 ppm and S–CH_2 at 3.2 ppm. In the spectrum of zinc complex, the carboxyl proton of thiazole moiety disappeared and the position of amide NH proton undergoes a slight downfield shift due to coordination with Zn(II) ion. It is indicated that the drug molecule is coordinated with zinc ion through amide nitrogen and carboxyl oxygen atoms, respectively.

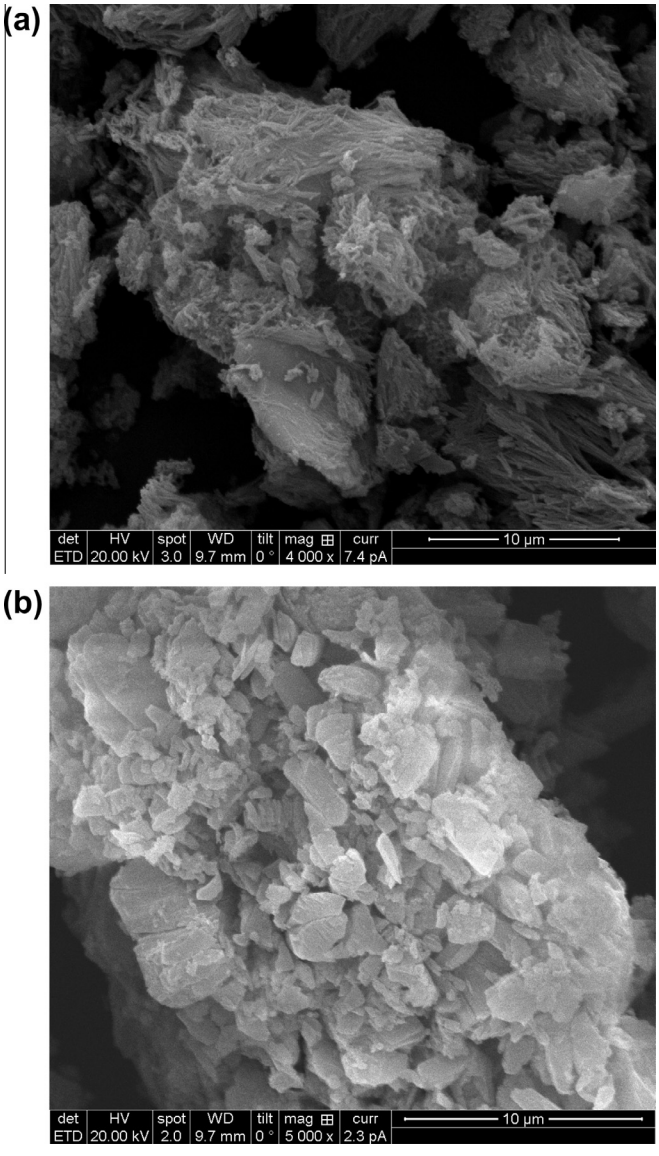

Figure 2 SEM images of drug (a) and copper complex (b).

3.4. ESR spectra

ESR spectrum of the cefixime–copper complex was recorded in DMSO at 300 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 was also evident from the absence of a half filled signal observed in the ESR spectrum at 1600G due to the $\Delta ms = \pm 2$ transition, ruling out any Cu–Cu interaction (Farmer and Urbach, 1974).

In the cefixime–Cu complex, the observed trend of g values is,

$$g||(2.21) > g \perp (2.04) > g_e(2.0036)$$

indicated that the unpaired electron is localised in the $d_x^2 - y^2$ orbital of the Cu(II) ion Ray and Kaufiman, 1990. The $A_{||}$ and $A_{\perp}$ values in the order $A|| (150 > A_{\perp} (36.5)$ also indicate that the complex has a square planar geometry and the system is axially symmetric (Raman et al., 2004).

Molecular orbital coefficients α^2 (inplane σ bonding), β^2 (in plane π bonding) and γ^2 (out-plane π bonding) were calculated using equations below (Benial et al., 2000).

$$\alpha^2 = -(A||/0.036) + (g|| - 2.0036) + 3/7(g \perp -2.0036) + 0.04 \quad (1)$$

$$\beta^2 = (g|| - 2.0036)E/ - 8\lambda\alpha^2 \quad (2)$$

$$\alpha^2 = (g \perp -2.0036)E/ - 2\lambda\alpha^2 \quad (3)$$

If the value of $\alpha^2 = 0.5$, it indicates complete covalent bonding, while the value of $\alpha^2 = 1.0$ suggests complete ionic bonding. In the present study, the observed α^2 value is 0.7125 which indicates that the complex has some covalent character in the ligand environment. The observed β^2 and γ^2 values of 1.256 and 0.7321 indicate that there is an interaction in the out of plane π bonding. The observed values of $k_{||}$ (0.82) > $K_{\perp}$ (0.533) imply a greater contribution from the out of plane π bonding in metal–ligand π bonding.

3.5. SEM study

The morphology and particle size of the metal–cefixime complexes have been illustrated by the scanning electron micrography (SEM). Fig. 2a and b depicts the SEM photographs of the copper–cefixime complexes and free cefixime. We have observed that there is a different morphology of the synthesised complexes in the pictograph. A rock like shape is observed in the Cu(II) complex. All other complexes were observed with a similar SEM behaviour.

On the basis of infrared spectral characteristics, elemental analysis, and magnetic susceptibility measurements, the structure of metal complexes are shown in Fig. 3.

3.6. Cyclic voltammetry

The cyclic voltammogram of 0.1 mM copper complex in the absence and presence of CT DNA is shown in Fig. 4. The cathodic peak potential (*Epc*) and the anodic peak potential (*Epa*) in the absence of DNA are −0.436 and −0.862 V, respectively. The separation of the anodic and cathodic peak potentials (Δ*E*), is 206 mV, and the ratio of cathodic to anodic peak currents, *ipc*/*ipa*, is 1.08, indicating a quasi- reversible redox process. The observed $E_{1/2}$ potential of copper complex is

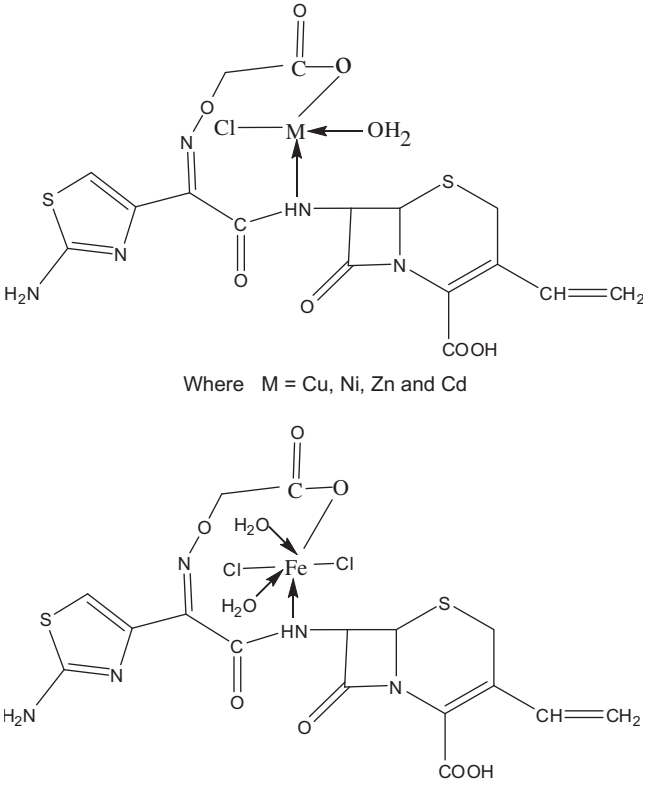

Figure 3 Structure of metal complexes.

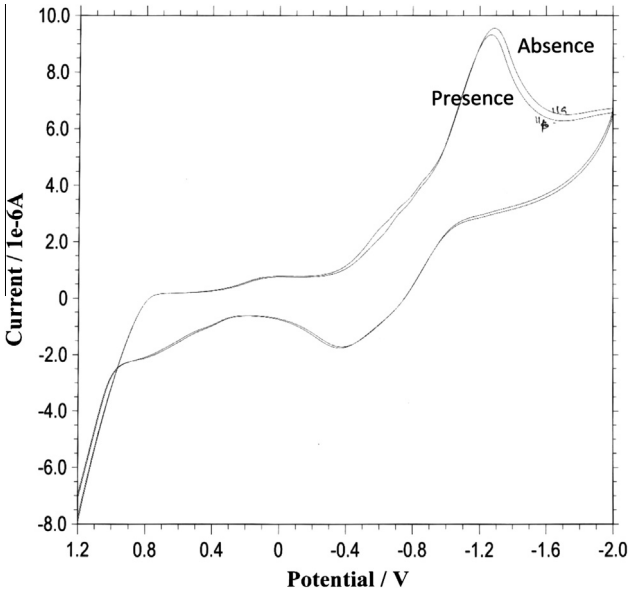

Figure 4 Cyclic voltammogram of copper complex in the absence and presence of CT DNA.

0.22 V in the absence of DNA. In the presence of DNA in the same solution of complex causes a negative shift in $E_{1/2}$ of 0.14 V. The decrease in peak currents can be explained in terms of an equilibrium mixture of free and DNA-bound copper (II) complex to the electrode surface.

Table 2 Minimum inhibitory concentration (MIC) (μg/L) of compounds against bacterial species.

Compound	*E. coli*	*K. pneumoniae*	*S. typhi*	*P. aeruginosa*	*S. aureus*
Drug	*48*	*40*	*40*	*42*	*43*
Cef-Cu(II)	46	38	39	42	42
Cef-Ni(II)	44	39	39	41	42
Cef-Zn(II)	45	38	38	40	43
Cef-Cd(II)	46	37	36	40	40
Cef-Fe(III)	40	35	34	39	39

Table 3 Minimum inhibitory concentration (MIC) (μg/L) of compounds against fungal species.

Compound	*A. niger*	*R. stolonifer*	*A. flavus*	*R. bataicola*	*C. albicans*
Drug	38	36	32	30	32
Cef-cu(II)	34	35	30	29	30
Cef-Ni(II)	32	35	31	30	32
Cef-Zn(II)	35	34	32	31	31
Cef-Cd(II)	34	34	31	31	32
Cef-Fe(III)	31	32	30	28	29

3.7. Antimicrobial studies

The drug (cefixime), metal complexes, and DMSO solvent were screened separately for their antimicrobial activity against bacterial and fungal species. The microbial results are summarised in Tables 2 and 3. The antimicrobial studies suggested that the drug is biologically active. Its metal complexes showed significantly enhanced antibacterial and antifungal activity against microbial strains in comparison to the free drug. Positive controls (Standard drug, parent moiety) produced significantly sized inhibition zones against the tested bacteria and fungi; however, the negative control (DMSO) produced no observable inhibitory effect against any of the test organisms.

A comparison of the MIC values of the drug and its metal complexes indicates that complexes exhibit a higher antimicrobial activity than the free ligands. Such increased activity of the complexes can be explained on the bases of overtones concept (Anjancyula and Rao, 1986) and Tweedy's chelation theory (Prabhakaran et al., 2004). According to the overtone's concept of cell permeability the lipid membrane that surrounds the cell favours the passage of only the lipid-soluble materials. Due to this liposolubility becomes one of the important factor in controlling the antimicrobial activity. On chelation, the polarity of the 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 delocalisation of π-electrons over the whole chelate ring and, enhances the lipophilicity of the complexes. This increased lipophilicity enhances the permeation of the complexes into liquid membranes and blocking of the metal binding sites in the enzymes of micro organisms. The complexes also disturb the respiration process of the cell and then block the synthesis of the proteins that restricts the growth of the organism.

In general, metal complexes are more active than the parent compound as they may serve as the principal cytotoxic species. Thus exhibiting their broad spectrum nature can also be used in pharmaceutical industry for the betterment of mankind, as a new antimicrobial agent, after testing its toxicity in human beings.

4. Conclusion

Mononuclear complexes of cefixime were synthesised and characterised by various physico-chemical techniques. The overall geometry around respective metal ions has been drawn on the basis of electronic spectral and magnetic moment studies. The comparative antibacterial activity on the drug, cefixime and its metal complexes show considerable antimicrobial activity. The metal complexes have more antimicrobial activity than the drug, cefixime. It is hoped that the synthesis of these complexes may be recommended for a new line of search for new antibiotics.

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