SYNTHESIS, CHARACTERISATION AND BIOLOGICAL STUDIES OF METAL COMPLEXES OF CEFIXIME

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Abstract--*Metal complexes of cefixime such as* [Cu(cef)(H_2O)Cl].H_2O, [Zn(cef)(H_2O)Cl].H_2O, [Ni(cef)(H_2O)Cl].$5H_2O$ and [Fe(cef)(H_2O)$_2Cl_2$].$4H_2O$ *have been synthesized and characterised by elemental analysis, IR ,UV-Visibe, ESR, NMR,XRD spectrscopic techniques. Thermal analysis was studied by TGA, DSC method. Morphological studies were carried out by scanning electron microscopic method. Furthur the complexes were screened for their antimicrobial property. From the spectral studies a square planar geometry was proposed for the copper complex. In conclusion, prepared complexes showed enhanced biological activities than the parent drug that might be of interest for future research*.

Keywords;Metalcomplexes.ligands,cefixime,antimicrobial**.**

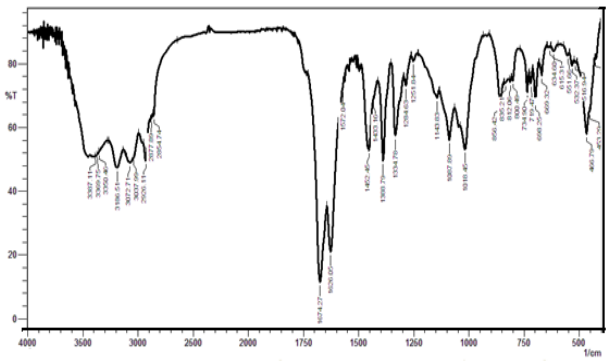
1. Synthesis of metal complexes

Equimolar ethanolic solutions of cefixime(drug) and metal salts ($CuCl_2.2H_2O$, $ZnCl_2.2H_2O$, $NiCl_2.6H_2O$, $FeCl_3.6H_2O$) were mixed at room temperature. Then, the pH of the solution mixture was adjusted to 8.0 using 0.5M NaOH. The precipitated complexes were filtered off, washed with ethyl alcohol and dried in vacuum. The molar conductance values of the metal complexes (measured in 10^{-3} M DMSO) are in the range of 3–6 Ω^{-1} cm^2 mol^{-1} indicating the non electrolytic nature. The purity of metal complexes has been checked by TLC. The complexes were formulated as[Cu(cef)(H_2O)Cl].H_2O,[Zn(cef)(H_2O)Cl].H_2O, [Ni(cef)(H_2O)Cl].$5H_2O$ and [Fe(cef)(H_2O)$_2Cl_2$].$4H_2O$.

2. Results and discussion

2.1 IR spectrum

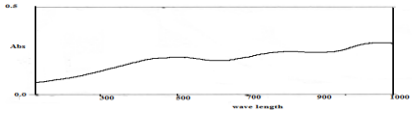
The IR spectrum of the free drug was compared with those of the metal complexes in order to ascertain the binding mode of the drug to metal ion in the complexes (Fig 1). The lactam band appears at 1766 cm^{-1} in the free cefixime while in the copper complex also it appears at 1766 cm^{-1} suggesting 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 it appears 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 are 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} [1]. In the spectra of metal complexes a broad band in the region 3300–3420 cm^{-1} indicates the presence of coordinated water molecules. In the case of [Fe(cef)(H_2O)$_2Cl_2$].$4H_2O$ 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, carboxylic oxygen atom of thiazole moiety and oxygen atom of water moleculesrespectively.



Fig; 1. IR spectrum of [Fe(cef)(H_2O)$_2Cl_2$].$4H_2O$

2.2 Electronic Spectrum

The electronic spectrum of Cu–cefixime complex (Fig 2) shows a broad absorption band between 600–700 nm which is assigned to $^2B_1g \rightarrow {}^2A_1g$ transition, indicating a square planar geometry of Cu(II)complex [2]. 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 $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ transition for tetrahedral geometry.Theelectronicspectrumof [Fe(cef)(H_2O)$_2Cl_2$].$4H_2O$ complex shows three bands at 983,755, and 506 nm which are assigned to $A_2g(F) \rightarrow T_2g(F)$, $A_2g(F) \rightarrow T_1g(F)$, and $A_2g(F) \rightarrow T_1g(P)$ transitions, respectively. The results were in accordance with octahedral geometry for Fe(III)–cefiximecomplex[3].



Fig;2**.** Electronic spectrum of[Cu(cef)(H_2O)Cl].H_2O complex

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2.3 ^{1}H NMR spectrum

The ^{1}H NMR spectrum of the zinc complex in $CDCl_3$(Fig 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 ($CH=CH_2$) at 4.5 ppm as a triplet and $CH=CH_2$ 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 11.1 and 12.4 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 amidenitrogenandcarboxylicoxygenatomrespectively.

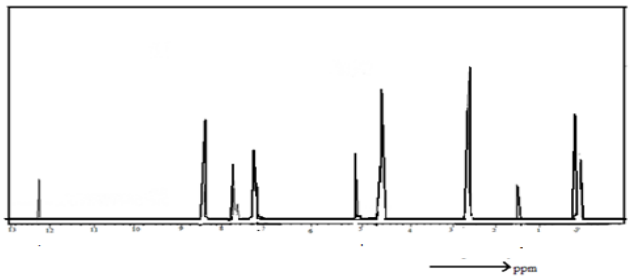
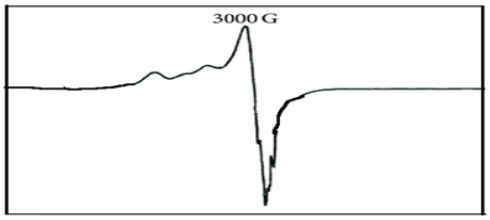


Fig ;3. ^{1}H NMR spectrum of [Zn(cef)(H_2O)Cl].H_2O

2.4 ESR spectrum

ESR spectrum of the [Cu(cef)(H_2O)Cl].H_2O complex (Fig 4) 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 $\Delta ms = \pm 2$ transition, ruling out any Cu–Cu interaction. In the cefixime–Cu complex, the observed trend of $g_{\|}$(2.354) > $g_{\perp}$ (2:256) > ge (2.0036) indicated that the unpaired electron is localised in the d_{x2-y2} orbital of the Cu (II) ion [4]. 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 [5]. Molecular orbital coefficients α^2 (inplane σ bonding), β^2(in plane π bonding) and γ^2 (out-plane π bonding) were calculated using equation [6]. 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. 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.



Fig;4. ESR spectrum of [Cu(cef)(H_2O)Cl].H_2O at 300K

2.5 XRD studies

The XRD pattern of the metallo drug [Cu(cef)(H_2O)Cl].H_2O was studied in the 2θ range of 5-35^{o} and the crystalline size of the complex (Fig 5) was calculated from Scherer's formula. From the observed XRD pattern the average crystalline size for the metallo drug was found to be 68 nm indicating that it is nanocrystalline in nature

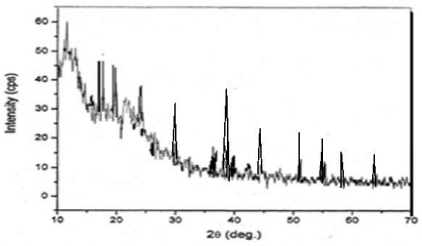


Fig ;5. Powder XRD pattern of [Cu(cef)(H_2O)Cl].H_2O

2.6 TGA studies

The metal complex [Cu(cef)(H_2O)Cl].H_2O undergoes three stages of decomposition. The first stage of decomposition takes place at 135.2^{o}C corresponding to 3.2% weight loss. (Fig 3.1.7). From 200-300^{o}C there is a decrease in weight loss as indicated by the sudden fall noted in the TG curve, this in turn indicates the stability of the metal complex. The second stage of decomposition corresponds to 88.23% weight loss. The decomposition in the third stage takes place at 491.3^{o}C corresponding to 97. 37% weight loss. 50% decomposition occurs at 266.4^{o}C [7,8].The decomposition fragments are $C_9H_5O_3SN$ and $C_4H_4N_4O_3S$. The weight of residue is 16.3%

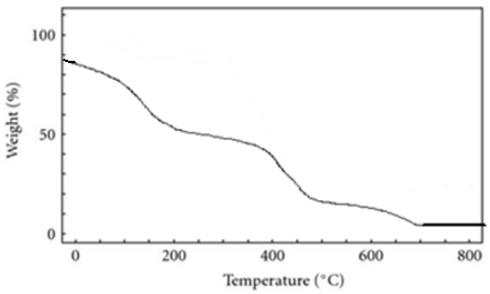


Fig ;6. TGA of [Cu(cef)(H_2O)Cl].H_2O

2.7 DSC studies

DSC behaviour of [Fe(cef)(H_2O)$_2$$Cl_2$].4$H_2O$ has been studied in the temperature range of 0-400^{o}C. The metal complex (Fig 6) shows the single Tg at 151.4^{o}C. The [Fe(cef)(H_2O)$_2$$Cl_2$].4$H_2O$complex shows a sharp endo thermal peak at 165^{o}C indicating the melting of the complex. The endothermic peak noted at 236.7^{o}C can be attributed to the decomposition of

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the complex. Broad exothermic peak observed at 290°C and 391°C can also be due to the decomposition of the complex [9,10].

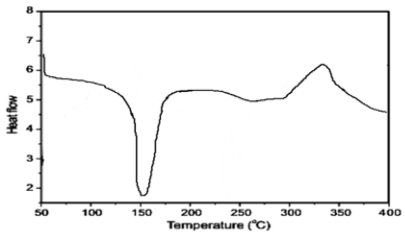
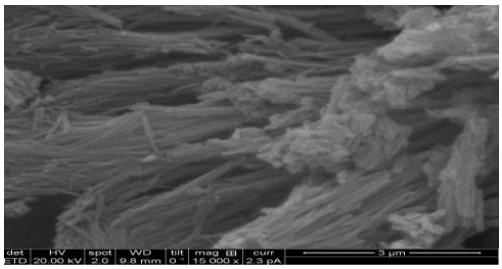


Fig ;7. DSC pattern of $[Fe(cef)(H_2O)_2Cl_2].4H_2O$

2.8 SEM studies

From the SEM photographs of the $[Cu(cef)(H_2O)Cl].H_2O$ complex (Fig7) and (Fig 8) free cefixime, a different morphologies of the synthesised complexes were noticed. A rock like shape is observed intheCu(II)complex.



Fig;8. SEM image of ceixime drug

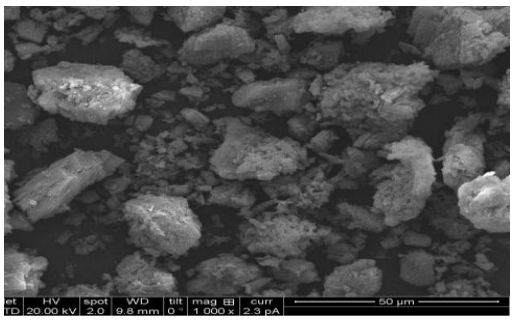


Fig ; 9. SEM image of $[Cu(cef)(H_2O)Cl].H_2O$

2.9 FAB mass spectrum

In the FAB mass spectrum of $[Cu(cef)(H_2O)Cl].H_2O$ complex (Fig 10) the molecular ion peak was observed at m/z 557 which is in agreement with the molecular weight (557) of the proposed structure. The spectrum showed the most abundant peak at m/z 453,which is assigned to the ligand. The spectrum showed peaks at m/z 161 and m/z 99, which are assigned to propiolactum and thiazole moieties. A similar type of fragmentation pattern was observed for other complexes.

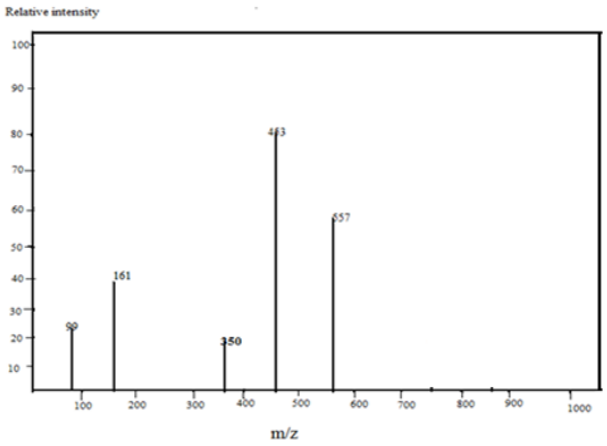


Fig ;10. Mass spectrum of $[Cu (cef)(H_2O)Cl].H_2O$

3. Antimicrobial Studies

3.1 Introduction

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) [11]. Some other organisms are also showing drug resistance like *M.tuberculosis* (Strain no: H_{37}Rv 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 *Klebsiellea pneumonia* is another drug resistant bacteria. This also extends to other gram negative organisms like *Escherichia coli, Shigella flexeneri, Pseudomonas aeruginosa,* and *Salmonella typhi* and Gram-positive *Bacillus subtilis* bacterial strains. Even some fungal pathogens are also showing the resistance feature against drugs like *Candida albicans, Aspergillus flavus, Fusarium solani,* and *Candida glaberata.*

Many metal complexes have powerful antimicrobial activities and are already in common day-to-day use in medicinal field such as silver bandages for treatment of burn, zinc antiseptic creams, bismuth drugs for the treatment of ulcers and metal clusters as anti-HIV drugs. The potential for further development of metal-based drugs as antimicrobial agent [12,13] is enormous and also of great importance with the evolution of drug-resistant bacteria and threats from a range of viral diseases. The discovery and development of antibiotics is among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. Metal-based drugs represent a novel group of antifungal agents with potential applications for the control of fungal infections. This inspires synthetic chemists to search for new metal complexes for bioactive compounds and zinc in particular has attracted the researchers. The field of macrocylic chemistry of metals is developing very rapidly because of its applications and importance in the area of coordination chemistry [14].

3.2 Results and discussion

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Antibacterial activity of the ligands/drugs and their complexes were tested against the bacterial species *Staphylococcus aureus, Escherichia coli, Klebsiella pneumaniae, Proteus vulgaris,* and *Pseudomonas aeruginosa* by Kirby Bauer Disc diffusion method [15]. The ligands and their complexes were also tested against the fungal species *Aspergillus niger, Rhizopus stolonifer, Aspergillus flavus, Rhizoctonia bataicola,* and *Candida albicans,* cultured on potato dextrose agar medium and also performed by the disc diffusion method. 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 [16] against bacterial and fungal pathogens. 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 [17] and Tweedy's Chelation Theory [18]. 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. The variation in the effectiveness of different compounds against different organisms depends either on the impermeability of the cells of the microbes or on the differences in the ribosomes of microbial cells.

From the observation of MIC values, the higher inhibition of microbial growth by metal complexes is due to the presence of uncoordinated hetero atoms and carboxylic moieties. In the complexes, the ligands have some uncoordinated donor atoms which enhance the activity of the complexes by bonding with trace elements present in microorganisms. This may combine with the uncoordinated site and inhibit the growth of microorganisms. The biospectrum of cefixime and its metal complexes against bacterial species and fungal species are shown in chart 1 and chart 2.

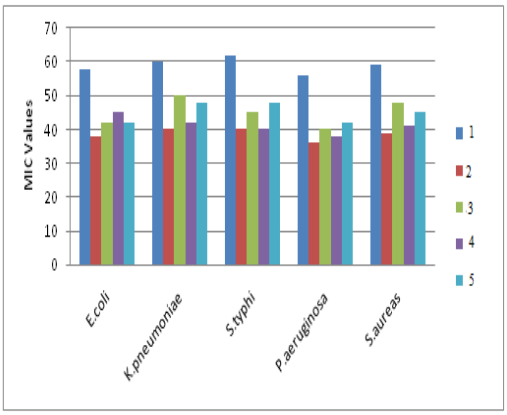

Chart 1; Minimum inhibitory concentrations of cefixime and its metal complexes against bacterial species (μg/ml)

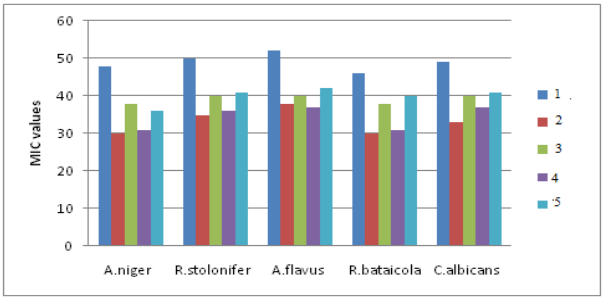

CHART 2 ; MINIMUM INHIBITORY CONCENTRATIONS OF CEFIXIME AND ITS METAL COMPLEXES AGAINST FUNGAL SPECIES (μG/ML)

4. Conclusion

The study of the reaction between the transition metal 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 cation of important biological role, in studying 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, ^{1}HNMR, and mass spectrum.

Fig 11;Geometry of the metal complex of cefixime M = Cu(II) ion

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