Evaluation of antibacterial activity of macrocyclic metal complexes derived from dihydrazone and diketone
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ABSTRACT
Synthesis of macrocyclic metal complexes of Cr(III) and Fe(III) by the condensation of benzil dihydrazone with 1H-indole-2, 3-dione. The metal complexes were characterized by elemental analysis, molar conductance, magnetic susceptibility, thermal analysis, ESR, infrared, 1H NMR and UV-visible spectroscopy. On the bases of these studies, a five-coordinate square pyramidal geometry is proposed for all the metal complexes. In vitro antibacterial activity of macrocyclic metal complexes exhibited good results.

Introduction
Although, it is well know that coordination of transition metals with macrocyclic enhance their biological activity. Metal reduced polarity of complexes and facilitate easy penetration into lipid membranes of bacterial cell and metal complex inhibit the protein synthesis by blocking the metal binding sites of the pathogen’s enzymes exhibit antibacterial activity [1]. Macrocyclic metal (II) complexes have various biological activities like antibacterial and antifungal [2, 3], antiviral, anticancer [4], radio-immunotherapeutic agents [5], DNA nuclease activity and detection of tumor lesions [6]. Here we have reported new macrocyclic complexes of Cr(III) and Fe(III) obtained by template method from benzil dihydrazone and 1H-indole-2,3-dione. In vitro antibacterial screening of metal complexes were done. Some of the complexes exhibited remarkable antibacterial activity.

Experimental

Materials and method
Analytical reagent grade chemicals were used. 1H-indole-2,3-dione, benzil, hydrazine hydrate, Cr(III) and Fe(III) salts, ciprofloxacin and solvents were purchased from Sigma-Aldrich Merck and Himedia.

Synthesis
Benzil dihydrazone was synthesized by refluxing of benzil and hydrazine hydrate in ethylene glycol for 2 h according to reported method [7]. The metal complexes were synthesized by refluxing of a 50 mL (20 mmol) methanolic solution of benzil dihydrazone, 50 mL (20 mmol) methanolic solution of 1H-indole-2,3-dione and 50 mL (10mmol) of Cr(III) or Fe(III) salts in 2:2:1 molar ratio for about 8 h. Glacial acetic acid (3-4 drops) was added to reaction mixture as catalyst. The product was then filtered and washed with methanol and dried in vacuum desiccator. Yield obtained about 66-70% (Fig.1 and Fig.2).

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Where M = Cr(III) and Fe(III). X = Cl\(^-\) and NO\(_3\)^-.

Fig. 2: Synthesis of macrocyclic metal (III) complex

**Analytical and physical measurements**

The C H N analysis was carried out with Euro, Elemental Analyzer. The infrared spectra were recorded with Thermo Scientific Nicolet iS50 FTIR Spectrometer in the range 4000-4000 cm\(^{-1}\). UV-Visible spectra were recorded by LAMBDA 25 PerkinElmer spectrophotometer. \(^1\)H NMR spectra were recorded with Bruker NMR spectrometer at 400 MHz. The metal content was determined with atomic absorption spectrophotometer, PG instruments. Magnetic susceptibility was carried out with Gouy balance. Molar conductivity was measured with digital conductivity meter, Hach. Thermal analysis was carried out with TA instruments. ESI mass spectra (m/z) were recorded with Agilent mass spectrometer. The X-band EPR spectra was recorded with ESR-JEOL spectrometer at room temperature.

**In vitro antibacterial activity**

In vitro antibacterial activity of Cr(III) and Fe(III) complexes were carried out against some bacterial strains i.e. *B. subtilis*, *S. aureus*, *P. aeruginosa* and *E. coli* by agar well diffusion method [8]. The overnight grown bacterial cultures were adjusted to 0.5 McFarland standard turbidity, which is approximately equal to 1.5 x 10\(^8\) Cfu/mL. The Plates were prepared by 20 ml Mueller Hinton agar media and wait for 10 min than 100 \(\mu\)L bacterial culture were spread over it. In agar medium 8 mm wells were bored with sterilized cork borer and filled with 100 \(\mu\)L of test compound with concentration of 1mg/mL in DMSO. All the plates were incubated at 37 °C for 24 h. The DMSO was used as negative control and ciprofloxacin as positive control. The zone of growth inhibition was measured.

**Results and Discussion**

According to different physicochemical studies dark coloured octaaza-macro cyclic complexes of Cr(III) and Fe(III) were obtained and found soluble in dimethyl sulphoxide (DMSO) and dimethyl formamide (DMF). Molar conductivity (10\(^2\)M) of complexes were measured in DMSO and found electrolytic in nature.

**Elemental Analysis**

The C H N analysis results of metal complexes agree well with the metal and elemental content calculated values (Table 1).
Infrared Spectroscopy
The pair of bands at 3248 and 3340 cm\(^{-1}\) for ν(NH\(_2\)) straching vibrations of benzil dihydrazone and ν(C=O) vibrations at 1730 cm\(^{-1}\) of 1H-indole-2,3-dione were absent in infrared spectrum of metal complexes. A strong medium band at 3188-3218 cm\(^{-1}\) of ν(NH) and strong band appeared at 1590-1596 cm\(^{-1}\) assigned to and ν(C=N) vibrations in the spectra of metal complexes [9]. The lower value of ν(C=N) indicates coordination of metal atom with azomethine nitrogen [10]. A macrocyclic frame was formed by the condensation of carbonyl oxygen (C=O) and amino (NH\(_2\)) groups [3]. The bands present at 3012-3053 cm\(^{-1}\) and 1497-1564 cm\(^{-1}\) may be assigned to ν(C-H) and ν(C=C) groups of aromatic rings respectively [10-11]. The far IR spectra show bands at 460–482 cm\(^{-1}\) assigned to ν(M–N) vibrations in all the complexes [12-13].

\(^1\)H NMR spectroscopy
The \(^1\)H NMR spectra of macrocyclic metal complex showed multiplets at 7.2 – 7.8 ppm may assigned to protons of aromatic rings of 1H-indole-2,3-dione [14-15]. A singlet appeared at 10.9 ppm may assigned to single proton (-NH) of isatin [16].

Magnetic studies and electronic spectra
Electronic absorption spectra of Cr(III) and Fe(III) complexes were recorded in DMSO and magnetic moment measurement of complexes by the Gouy balance at room temperature. The magnetic moment measurement and absorption bands value agree well with the octahedral geometry for all the complexes [17-18]. The electrolytic nature of the complexes supported the structure as shown in Table 2.

Thermal Analysis
Thermogravimetric (TG/DTA) analyses of Cr(III) and Fe(III) complexes was carried out from room temperature to 700 °C at 10 °C/min. Thermogram of metal complexes shows no weight loss up to 270 °C confirming the absence of any coordinated water molecules in the complex. The complex shows weight loss in a single stage around 270-450 °C. The weight loss is corresponding to sublimation of chloride ions and macrocyclic ligand moieties. In the range of 500–600 °C an air stable metal oxide is formed. The thermal analysis results agree well with the composition of the metal complexes.

Mass spectra
ESI mass of \([\text{Cr(C}_{44}\text{H}_{30}\text{N}_{10})\text{Cl}_2]\)Cl and \([\text{Fe(C}_{44}\text{H}_{30}\text{N}_{10})\text{Cl}_2]\)Cl exhibited molecular ion (m/z) peak at 857.13 and 860.97 amu in agreement with the results obtained by microanalysis. The obtained molecular ion (m/z) peaks confirm the proposed structure of metal complexes.

Antibacterial activity results
The result of agar well diffusion method shows that Cr(III) metal complexes exhibited remarkable antibacterial activity higher than Fe(III) complexes against Gram positive and Gram negative bacteria. Ciprofloxacin was used as broad spectrum standard antibiotic (Table 3).

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### Table 1: Analytical and physical data of complexes

<table>
<thead>
<tr>
<th>S. N.</th>
<th>Compound</th>
<th>Colour</th>
<th>Melting point (°C)</th>
<th>Mol. mass</th>
<th>Elemental analysis, calcld. (found)%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>1</td>
<td>[Cr(C(<em>{44})H(</em>{30})N(_{10})]\text{Cl}_2]\text{Cl}</td>
<td>Orange</td>
<td>223</td>
<td>857.13</td>
<td>6.07</td>
</tr>
<tr>
<td>2</td>
<td>[Cr(C(<em>{44})H(</em>{30})N(_{10})]\text{(NO}_3)\text{Cl}_2]</td>
<td>Orange</td>
<td>236</td>
<td>936.78</td>
<td>5.55</td>
</tr>
<tr>
<td>3</td>
<td>[Fe(C(<em>{44})H(</em>{30})N(_{10})]\text{Cl}_2]</td>
<td>Dark brown</td>
<td>270</td>
<td>860.97</td>
<td>6.49</td>
</tr>
<tr>
<td>4</td>
<td>[Fe(C(<em>{44})H(</em>{30})N(_{10})]\text{(NO}_3)\text{Cl}_2]</td>
<td>Dark brown</td>
<td>281</td>
<td>940.63</td>
<td>5.94</td>
</tr>
</tbody>
</table>

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### Table 2: Magnetic studies and electronic spectral data

<table>
<thead>
<tr>
<th>Compound</th>
<th>Electronic spectral bands (cm(^{-1}))</th>
<th>Assignment</th>
<th>Magnetic moment (B.M.)</th>
<th>Molar conductance (ohm(^{-1}) cm(^2) mol(^{-1}))</th>
<th>Geometric structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr(III) complexes</td>
<td>17480–17590, 24648–25650, 28674–30408</td>
<td>(^4)A(<em>{2g} \rightarrow )T(</em>{2g}) (F), (^4)A(<em>{2g} \rightarrow )T(</em>{1g}) (F), (^4)A(<em>{2g} \rightarrow )T(</em>{1g}) (P)</td>
<td>4.36 – 4.48</td>
<td>80–98</td>
<td>Octahedral</td>
</tr>
<tr>
<td>Fe(III) complexes</td>
<td>16865–16890, 23508–23585, 28761–28812, 32901–33010</td>
<td>(^6)A(<em>{1g} \rightarrow )T(</em>{2g}) (G), (^6)A(<em>{1g} \rightarrow )T(</em>{1g}) (G), (^6)A(<em>{1g} \rightarrow )T(</em>{2g}) (D), (^6)A(<em>{1g} \rightarrow )T(</em>{1g}) (P)</td>
<td>5.82 – 5.88</td>
<td>88–100</td>
<td>Octahedral</td>
</tr>
</tbody>
</table>
Conclusion
On the basis of various physicochemical studies octaaza-macrocyclic octahedral structure is proposed of all the metal complexes. Thermogravimetric analysis data of metal complexes found in agreement well with the proposed structure. Synthesized macrocyclic Cr(III) complexes exhibited remarkable antibacterial activity. Although it has been reported that metal enhance penetration rate into lipid membranes of bacterial cell wall [1], however further studies for better understanding about the mechanism of antibacterial action and significance of research work may required.

Acknowledgements
Authors are thankful to Department of Applied Chemistry, School of Vocational studies and Applied Sciences, Gautam Buddha University, Greater Noida for the necessary research facilities.

Abbreviations
CFU : Colony forming unit
B.M. : Bohr Magneton
DMF : N,N-dimethylformamide
DMSO: Dimethylsulphoxide
amu : Atomic mass unit

References

15. Singh DP, Kumar R, Kamboj M, Grover V, Jain K. Template synthesis and characterization of N6 12-membered macrocyclic complexes derived from isatin

Table 3: Antimicrobial activity by agar well diffusion method

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compounds</th>
<th>Zone of growth inhibition, diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B. subtilis</td>
</tr>
<tr>
<td>1</td>
<td>[Cr(C4H8N2O4Cl)]Cl</td>
<td>22.8</td>
</tr>
<tr>
<td>2</td>
<td>[Cr(C4H8N2O4)3NO3]Cl2</td>
<td>27.1</td>
</tr>
<tr>
<td>3</td>
<td>[Fe(C4H8N2O4)3Cl]Cl</td>
<td>19.0</td>
</tr>
<tr>
<td>4</td>
<td>[Fe(C4H8N2O4)(NO3)2NO3]</td>
<td>19.3</td>
</tr>
<tr>
<td>5</td>
<td>Ciprofloxacin</td>
<td>30.0</td>
</tr>
</tbody>
</table>

Research Article


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