INTRODUCTION

The metal-complexes having macrocyclic moiety get high attention in research field during the years of last and present century due to resemblances with many natural complex systems such as porphyrins [1] and cobalamines [2]. The synthesis of macrocyclic compounds is an important area of chemistry due to their applications for instance their role in antibacterial [3,4], antifungal [5], water-treatment [6,7] and antitumor [8] activities. Specifically, nitrogen-containing macrocyclic complexes make them more interesting in scientific, technical and medical fields due to their potential applications in these fields [9-13]. Thus, synthesis and characterization of macrocyclic complexes as aza-macrocyclic have evolved as the main research area in recent years. Present work reported on the synthesis, characterization, and biological activities of aza-macrocyclic complexes of Fe(III) and Cr(III), which is derived from dimedone, dibenzoylmethane and 3,4-diaminotoluene. Additionally, the dye removal efficiency of the Fe(III) complexes was also evaluated as well.

EXPERIMENTAL

The analytical reagent grades of 3,4-diaminotoluene, dibenzoylmethane, dimedone, acetone, acetic acid and DMSO were purchased from Sigma-Aldrich and Loba Chemie whereas trivalent chromium and iron salts were acquired from Himedia and CDH, India. All of these chemicals employed in present work were of analytical grade and used without any further purification. The microbes Bacillus subtilis, Escherichia coli and Candida albicans were procured from the Microbial Type Culture Collection (MTCC); IMTECH (Chandigarh, India). The IR spectra were recorded on the MB-3000 FTIR spectrometer. The XEVO G2-XS QT mass spectrometer was used for obtaining the mass spectra of all complexes. Elemental analysis data was obtained from a EuroEA elemental analyzer, whereas electronic spectra were recorded on Perkin-Elmer Lambda UV Spectrophotometer. The CM-183 conductivity meter was used to measure the conductance of all the metal(III) complexes. The magnetic susceptibilities of all complexes were determined using a PAR-155 magnetometer. The powder X-ray diffraction
studies were conducted on an XPret Pro X-ray diffractometer. The gravimetric method was utilized to determine the metal contents of newly synthesized chromium complexes. The back titration method was adopted to determine the metal content of the iron complexes. The electrical melting point machine was employed to determine the melting point of complexes by using fine capillaries and are uncorrected.

**Isolation of metal(III) complexes:** All the metal(III) complexes were synthesized by using the template method. In this method, 3,4-diaminotoluene, dibenzoylmethane and dimedone were condensed in the presence of Cr(III) or Fe(III) metal salts. A metal adduct was formed by adding trivalent transition metal salt (10 mmol) to the methanolic solution of 3,4-diaminotoluene (20 mmol) and refluxing the mixture for 30 min. After that the methanolic solution of dibenzoyl methane (10 mmol) was added to the above solution and refluxed for a period 4-5 h (Scheme-I) with continuous stirring. The mixture was then refluxed again for 6-8 h after adding the methanolic solution of dimedone (10 mmol) (Scheme-I). The resultant mixture was then allowed to cool overnight, yielding dark-coloured precipitates. The precipitate was filtered, washed with diethyl ether and finally dried in vacuum oven for overnight.

**Complex 1:** [Fe(C_{37}H_{36}N_{4})(Cl)]Cl_{2}; Yield: 60%, dark red, 223 °C, m.w.: 698.92; molar conductance = 168 ohm⁻¹ cm² mol⁻¹; Anal. calcd. found (%): Fe, 7.99 (7.57); C, 63.58 (63.17); H, 5.19 (4.91); N, 8.02 (7.79).

**Complex 2:** [Fe(C_{37}H_{36}N_{4})(NO₃)](NO₃)₂; Yield: 68%, dark brown, 243 °C, m.w.: 788.58; molar conductance = 161 ohm⁻¹ cm² mol⁻¹; Anal. calcd. found (%): Fe, 7.17 (6.93); C, 57.08 (56.72); H, 4.66 (4.21); N, 12.59 (12.11).

**Complex 3:** [Fe(C_{37}H_{36}N_{4})(OAc)](OAc)₂; Yield: 58%, dark red, 214 °C, m.w.: 695.71; molar conductance = 144 ohm⁻¹ cm² mol⁻¹; Anal. calcd. found (%): Fe, 7.26 (7.04); C, 67.10 (66.79); H, 5.89 (5.37); N, 7.28 (6.89).

**Complex 4:** [Cr(C_{37}H_{36}N_{4})(Cl)]Cl₂; Yield: 63%, black, 229 °C, m.w.: 695.71; molar conductance = 159 ohm⁻¹ cm² mol⁻¹; Anal. calcd. found (%): Cr, 7.48 (7.13); C, 63.94 (63.21); H, 5.22 (5.02); N, 8.06 (7.59).

**Complex 5:** [Cr(C_{37}H_{36}N_{4})(NO₃)](NO₃)₂; Yield: 60%, black, 214 °C, m.w.: 774.73; molar conductance = 153 ohm⁻¹ cm² mol⁻¹; Anal. calcd. found (%): Cr, 6.71 (6.16); C, 57.36 (56.98); H, 4.68 (4.25); N, 12.66 (12.18).

**Complex 6:** [Cr(C_{37}H_{36}N_{4})(OAc)](OAc)₂; Yield: 54%, black, 231 °C, m.w.: 765.85; molar conductance = 140 ohm⁻¹ cm² mol⁻¹; Anal. calcd. found (%): Cr, 6.79 (6.21); C, 67.44 (67.24); H, 5.92 (5.47); N, 7.32 (7.03).

**Biological assay**

Test microorganisms: Three pathogenic strains of bacteria (two) and fungi (one) were used as test targets for the examination of biological activity of all synthesized macrocyclic complexes. One Gram-positive *i.e.* Bacillus subtilis, one Gram-negative bacterium strain *i.e.* Escherichia coli and one fungal strain *i.e.* Candida albicans were used for evaluation of antibacterial and antifungal activities of synthesized metal(III) complexes in the present study.

**Primary screening:** The agar-well diffusion technique was adopted to assess the antimicrobial properties of the synthesized metal(II) complexes [14]. Each petri-plate contained 20 mL of agar medium, which was then swabbed with 100 µL microbes of the test microorganisms and left for 15 min to allow adsorption. Wells were driven into the seeded agar plates using a sterile cork borer with an 8 mm diameter, and these were filled with 100 µL solution of each complex in DMSO. For 24 h, all the plates were incubated at 37 °C. The zone analyzer was used to determine the extent to which each synthesized metal(III) complex inhibited the development of the investigated microorganisms and that extent of inhibition can be termed as the zone of inhibition (ZOI). Ciprofloxacin and Amphotericin B were used as standard antibiotic and antifungal drug, respectively.

**Adsorption of colored effluents from aqueous solution:** A 100 mL stock solution of crystal violet dye of concentration 50 mg/L was prepared for the experimental study. Further, the solution was diluted to obtain solution of variable concentrations. A calibration curve was plotted using the absorbance values of the above prepared solutions (Fig. 1). The Na₂CO₃/
NaHCO₃ buffer was used to adjust the pH of the solution. Iron (III) complexes were added to the stock solution as well as the solutions of diluted concentrations. Additionally, 2.0 mL of H₂O₂ was added to the above mixtures and after 30 min, their absorbance values at maximum wavelength were measured [7]. The effect of amount of iron(III) complexes on the adsorption of coloured effluent, crystal violet dye from aqueous solution was investigated using variable amount of iron complexes and fixed concentration of dye solution at constant temperature. The percentage dye removal efficiency of Fe(III) complex can be calculated using the following equation:

\[
\text{Dye removal efficiency (\%)} = \frac{A_o - A_t}{A_o} \times 100
\]

RESULT AND DISCUSSION

The analytical data of Cr(III) and Fe(III) complexes suggested the general formula of all newly synthesized macrocyclic complexes as \([\text{M(C}_3\text{H}_3\text{N}_4\text{X})X_2]\); where \(\text{M} = \text{Fe}^{3+}\) and \(\text{Cr}^{3+}\) and \(X = \text{Cl}^-, \text{NO}_3^-\) and \(\text{CH}_3\text{COO}^-\). All the metal(III) complexes are soluble in DMSO whereas insoluble in other common organic solvents. The molar conductance values of all metal(III) complexes were found in the range 168-140 ohm⁻¹ cm² mol⁻¹ suggested the electrolytic nature. Moreover, the range of melting point of the metal(III) complexes suggested their thermal stability upto 223 °C beyond which the metal(III) complexes began to decompose. Further, the analytical data suggested the square pyramidal geometry around the central metal atom for all synthesized metal(III) macrocyclic complexes.

IR studies: The appearance of new band in the region 1640-1590 cm⁻¹ in IR spectra (Fig. 2) of all the metal(III) complexes may be attributed to the C=N stretching vibration and confirmed the formation of complexes as a result of condensation between –NH₂ groups and >C=O groups of the reactants [15]. The decrease in the stretching frequency of \(\nu(\text{C=}\text{N})\) displayed the shift of electrons of nitrogen atom of azomethine group to the coordinated metal ion [16]. The infrared spectra of all the metal complexes lacked a pair of peaks at 3300 and 3387 cm⁻¹ corresponding to –NH₂ groups that were observed in the IR spectrum of 3,4-diaminotoluene. Moreover, no peaks corresponding to carbonyl groups of dimedone and dibenzoyl methane were observed in the IR spectra of all complexes. This confirmed the condensation between diketo and diamino compounds. Addi-
tionally, all of the complexes exhibited a moderately intense band in the region of 3040-2840 cm⁻¹, which may be attributed to the stretching vibrations of sp³ C–H and sp³ C–H bonds [17]. The bands observed between 1195-1146 cm⁻¹ in the IR spectra of all the metal(III) complexes may be assigned to ν(C–N) vibrations.

**Electronic spectral studies**

**Iron(III) complexes:** The effective magnetic moments (µ eff.) of iron complexes were observed in the range 5.69-5.31 B.M. at room temperature, corresponding to five unpaired electrons, which is close to the predicted high spin values for these metal ions [18]. Three bands were found in the electronic spectra of iron complexes at 525-490, 383-345 and 260-220 nm (Fig. 3). These bands were attributed to the electronic transitions d₄ₓᵧ →dₓz, dₓᵧ →dₓz, and dₓᵧ →dₓz, respectively [4]. These bands showed a strong consistency with the already reported electronic spectral studies of Fe³⁺ complexes possessing the square pyramidal geometry.

**Chromium(III) complexes:** The effective magnetic moments (µ eff.) of Cr³⁺ complexes was found between 4.32 and 4.06 B.M. at room temperature, indicating that the metal ion includes three unpaired electrons [19]. In the electronic spectra of chromium complexes, bands at 690-630, 570-498 and 380-341 nm were observed (Fig. 4). These bands were attributed to the dₓz →dₓz, dₓz →dₓz, and dₓz →dₓz electronic transitions, respectively. The fourth transition band at 285-255 nm may be attributed to π →π* transition [4]. All of these spectral bands are compatible with the five-coordinated square pyramidal geometry of chromium(III) complexes. Therefore, it was proposed that chromium complexes also possessed square pyramidal geometry around the metal ion.

**Mass spectral studies:** The mass spectra of all the metal(III) complexes exhibit [M]⁺ ion peaks in addition to the other peaks. The m/z value of the molecular ion, i.e. [M]⁺, of the complexes provided information regarding their molecular weight. For instance, in C-4 complex m/z peak was observed at 695.37 (Fig. 5a), which is in close range to the calculated molecular weight of this complex i.e. 695.71. Similarly, in C-1 complex the m/z peak was observed at 698.58 (calcd. m.w.: 698.92) (Fig. 5b). C-2 complex showed m/z peak at 777.47 (calcd. m.w.: 778.58) (Fig. 5c), C-3 m/z peak showed at 768.19 (calcd. m.w.: 769.70), C-5 m/z peak showed at 774.61 (calcd. m.w.: 774.73) and C-6 m/z peak at 764.33 (calcd. m.w.: 765.85) (Fig. 5d) that showed close resembles with their molecular weights. The obtained results are in close agreement with the calculated molecular weight of all the metal(III) complexes and confirmed the proposed structure for these complexes.

**P-XRD studies:** The powder XRD analysis of synthesized macrocyclic complexes via template method was studied using an X-ray diffraction analysis. The synthesized metal(III) complexes were analyzed in the 2θ ranging from 4-85° at wavelength of 1.54 Å. Sharp peaks in the powder XRD pattern indicated that the complex is somewhat crystalline in nature. Computer software, FullProf suite was used to determine the lattice dimensions of all the synthesized metal(III) complexes [20]. It was found that the complexes C-1, C-2, C-3 and C-5 possessed monoclinic crystal system (Table-1), whereas complexes C-4 and C-6 followed the orthorhombic and triclinic crystal system, respectively.

**Adsorption behaviour of iron(III) complexes:** The adsorption behaviour of iron(III) complexes was evaluated using batch adsorption process. The effect of amount of iron(III) complexes (5-25 mg) on the adsorption of crystal violet dye was studied at constant temperature and pH 7-8. After 30 min of adsorption process, the solutions of reduced intensity were obtained. Furthermore, the calibration curve was used to measure the concentration of the solutions. These results revealed that the percentage removal efficiency of the synthesized iron(III) complexes increased with the increase amount of complexes (Fig. 6) added to the solution.

**Antimicrobial activity:** All the synthesized macrocyclic complexes were screened for their antimicrobial activity. These complexes show ZOI in the range 8-15 mm (Table-2). Most of the metal(III) complexes showed moderate antimicrobial activity against both bacterial and fungal strains, but complex C-2 possessed least antimicrobial activities against all the tested bacterial and fungal strains. However, complex C-1 exhibited highest antimicrobial activity among all metal(III) complexes.
It was also observed that all complexes were more active to inhibit the growth of *Escherichia coli* (Table-2) as compared to *Bacillus subtilis* and *Candida albicans*. These results may be obtained due to the compounds hydrophobic or lipophilicity character of these complexes which in turn assisted the diffusion across the lipid membrane in order to limit or hinder the viable growth of microbes [13]. Additionally, this may be explained with the aid of the chelation hypothesis as well. According to this hypothesis, an increase in hydrogen bonding or a reduction in the polarizability may lead to an increase in their lipophilicity, which in turn boosts its antimicrobial activity [21].

**Conclusion**

By using template approach, a new series of trivalent macrocyclic metal complexes was synthesized by the condensation of 3,4-diaminotoluene, dibenzoyl methane and dimedone in the presence of trivalent metal salts in 2:1:1:1 molar ratio. The
metal(III) complexes were characterized with various physiochemical and spectral studies, which suggested that synthesized metal(III) complexes have square pyramidal geometry around the metal ion. The PXRD analysis suggested that the complexes C-1, C-2, C-3 and C-5 possessed monoclinic crystal system, while the C-4 and C-6 complexes possessed the orthorhombic and triclinic crystal systems, respectively. Moreover, the antimicrobial activities of all the metal(III) complexes exhibited moderate potential to inhibit the growth of selected bacterial and fungal strain except complex C-2 that showed poor activity against *Escherichia coli* and no activity against *Bacillus subtilis* and *Candida albicans*.

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### CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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**TABLE-2**

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Diameter zone of inhibition (mm)</th>
<th>Fungal strain</th>
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<tbody>
<tr>
<td></td>
<td>Gram-negative</td>
<td>Gram-positive</td>
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<tr>
<td>C-1</td>
<td>15</td>
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<td>C-2</td>
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<tr>
<td>Ciprofloxacin</td>
<td>26</td>
<td>26</td>
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<tr>
<td>Amphotericin</td>
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