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# Synthesis, spectroscopic characterization, DNA interaction and antibacterial study of metal complexes of tetraazamacrocyclic Schiff base

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ABSTRACT

The template condensation reaction between benzil and 3,4-diaminotoulene resulted mononuclear 12-membered tetraimine macrocyclic complexes of the type, $[MLCl_2]$ [M = Co(II), Ni(II), Cu(II) and Zn(II)]. The synthesized complexes have been characterized on the basis of the results of elemental analysis, molar conductance, magnetic susceptibility measurements and spectroscopic studies *viz.* FT-IR, $^1H$ and $^{13}C$ NMR, FAB mass, UV–vis and EPR. An octahedral geometry has been envisaged for all these complexes, while a distorted octahedral geometry has been noticed for Cu(II) complex. Low conductivity data of all these complexes suggest their non-ionic nature. The interactive studies of these complexes with calf thymus DNA showed that the complexes are avid binders of calf thymus DNA. The *in vitro* antibacterial studies of these complexes screened against pathogenic bacteria proved them as growth inhibiting agents.



## 1. Introduction

In recent years, macrocyclic chemistry has received great attention for their unique properties such as biomimic functions [1,2], ionic and molecular recognition [3,4], as building block in supramolecular chemistry [5,6]. There is a continued interest in synthesizing macrocyclic complexes because of their potential applications in fundamental and applied sciences and importance in the area of coordination chemistry [7]. The chemical properties of macrocyclic complexes can be tuned to force metal ions to adopt unusual coordination geometry. The stability of macrocyclic metal complex depends upon a number of factors, including the number and type of donor atoms present in the ligand and their relative positions within the macrocyclic skeleton, as well as the number and size of the chelate rings formed on complexation [8]. The chemistry of Schiff base complexes has been an important and popular area of research due to their simple synthesis, versatility and diverse range of applications [9]. A considerable number of Schiff-base complexes have potential biological interest, being used as more or less successful models of biological compounds [10]. Macrocyclic Schiff base nitrogen donor ligands have received special attention because of their mixed hard–soft donor character, versatile coordination behavior [11,12], and for their biological activities *i.e.*, toxicity against bacterial growth [13], anticancerous [14] and other biochemical properties [15]. Polyaza macrocyclic Schiff bases have been studied as potential inorganic and organic cation receptors [16], electron transfer agents, homogeneous catalysts and DNA, RNA interacting agents [17]. Nitrogen donor macrocycles are an important class of compounds due to their prominent behavior of forming highly stable complexes with a variety of transition metal ions [18,19]. Macrocyclic compounds with basic nitrogen donors could also form protonated moieties, which can interact with simple as well as with more complex inorganic and organic anions [20]. The family of complexes with aza-macrocyclic ligands has remained a focus of scientific attention for many decades [21]. Transition metal complexes with their varied coordination environment and versatile redox and spectral properties offer immense scope for designing species that are suitable to bind and cleave the DNA [22]. Recently, there has been tremendous interest in studies related to the interaction of transition metal ions with nucleic acid because of their relevance in the development of new reagents for biotechnology and medicine [23].

In view of aforesaid applications we found it worthwhile to report a few 12-membered tetraazamacrocyclic complexes of type, $[MLCl_2]$ formed by [2+2] template condensation reaction of benzil and 3,4-diaminotoluene with Co(II), Ni(II), Cu(II) and Zn(II) ions. These synthesized complexes were tested for their *in vitro* antibacterial activity against some gram-positive bacterial strains *viz. Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus pyogenes*, *Staphylococcus epidermidis*, *Bacillus cereus*,

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*Corynebacterium xerosis* and gram-negative bacterial strains *viz. Escherichia coli*, *Klebsiella pneuomoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. The results obtained were compared with standard antibiotic: Ciprofloxacin (for gram-positive) and Gentamicin (for gram-negative) bacterial strains.

## 2. Experimental

### 2.1. Materials and methods

The chemicals, 3,4-diaminotoluene, benzyl and ethidium bromide (E. Merck) were used as received. The metal salts $MCl_2{\cdot}6H_2O$, M = Co(II) and Ni(II), $CuCl_2{\cdot}2H_2O$ and $ZnCl_2$ (all E. Merck) were commercially available pure samples. Methanol (AR) was used as solvent. The solid media namely nutrient agar no. 2 (NA) (M 1269S-500G, Himedia Labs Pvt. Ltd, Mumbai, India) was used for preparing nutrient plates, while nutrient broth (NB) (M002-500G, Himedia Labs Pvt. Ltd, Mumbai, India) was used for the liquid culture media. Highly polymerized calf-thymus DNA sodium salt (7% Na content) was purchased from Sigma. Other chemicals were of reagent grade and used without further purification. Calf thymus DNA was dissolved to 0.5% (w/w), (12.5 mM DNA/phosphate) in 0.1 M sodium phosphate buffer (pH 7.40) at 310 K for 24 h with occasional stirring to ensure formation of homogeneous solution. The purity of the DNA solution was checked from the absorbance ratio $A_{260}/A_{280}$. Since the absorption ratio lies in the range $1.8 < A_{260}/A_{280} < 1.9$, therefore no further deproteinization of DNA was needed.

### 2.2. Physical measurements

The elemental analyses were performed on Perkin-Elmer 2400 CHN Elemental Analyzer from the Micro-analytical Laboratory of Central Drug Research Institute (CDRI), Lucknow, India. The FT-IR spectra (4000–200 $cm^{-1}$) of the complexes were recorded as KBr/CsI discs on a Perkin-Elmer Spectrum RX-I spectrophotometer. $^1H$ and $^{13}C$ NMR spectra were recorded in DMSO-$d_6$ on Bruker Avance II 400 NMR spectrometer with $Me_4Si$ as an internal standard from SAIF, Punjab University Chandigarh. FAB mass spectra were recorded on a Joel SX-102 spectrometer. Metals and chloride were estimated volumetrically [24] and gravimetrically [25], respectively. The electronic spectra of the complexes in DMSO were recorded on a Cary 5E UV-VIS-NIR spectrophotometer at room temperature. EPR spectrum was recorded at liquid nitrogen temperature on E-112 ESR spectrometer using TCNE as the g-marker. Magnetic susceptibility measurements were carried out using a Faraday balance at 25 °C. The molar conductivities of the complexes ($10^{-3}$ M solutions in DMSO) were obtained on a Systronic type 302 conductivity bridge equilibrated at 25.00 ± 0.1 °C. Fluorescence measurements were performed on spectrofluorophotometer Model RF-5301PC (Shimadzu, Japan) equipped with a data recorder DR-3. A fixed concentration of complex solution (30 μM) was taken in a quartz cell and increasing amounts of calf thymus DNA solution was titrated. The excitation wavelength and emission range for [$CoLCl_2$], [$NiLCl_2$], [$CuLCl_2$], [$ZnLCl_2$] were (350 nm, 355–520 nm), (365 nm, 370–430 nm), (250 nm, 340–490 nm), and (250 nm, 375–480 nm), respectively. The path length of the sample was 1 cm with 5 nm slit at 310 K.

### 2.3. Synthesis of the complexes

#### 2.3.1. Dichloro [2,3:8,9-tetraphenyl-5,6:11,12-tetrabenzo-1,4,7,10-tetraazacyclododeca-1,3,7,9-tetraene] metal(II), [$MLCl_2$] [M = Co(II), Ni(II), Cu(II) and Zn(II)]

To a methanolic solution (20 ml) of metal salts (1 mmol), the methanolic solutions of both 3,4-diaminotoluene (2 mmol, 0.244 g) and benzil (2 mmol, 0.420 g) were added simultaneously with constant stirring. The reaction mixture was then refluxed for 4–5 h, resulting in a clear solution. The resultant solution was kept for evaporation at room temperature leading to the isolation of a microcrystalline product after a few days. The product was washed with methanol and dried in vacuum.

### 2.4. in vitro antibacterial activity

#### 2.4.1. Test microorganisms

Ten bacterial strains (six gram positive and four gram negative) were selected on the basis of their clinical importance in causing diseases in humans. These were obtained from Hi media Labs Pvt. Ltd., Mumbai, India and Microbial Type Culture Collection, Chandigarh, Punjab, India. The strains selected for the study are *S. aureus* (ATCC 29213), *S. mutans* (ATCC 25175), *Streptococcus pyrogenes* (MTCC 435), *S. epidermidis* (MTCC 435), *B. cereus* (MTCC 430), *C. xerosis* (ATCC 373) (gram-positive bacterial strains), *E. coli* (ATCC 25922), *K. pneuomoniae* (MTCC 109), *P. vulgaris* (MTCC 426) and *P. aeruginosa* (MTCC 424). These strains were screened for evaluation of antibacterial activities of the synthesized complexes.

#### 2.4.2. Primary screening

The antibacterial activity of the macrocyclic complexes was evaluated by agar well diffusion method [26]. All the microbial cultures were adjusted to 0.5 McFarland standards, which is visually comparable to a microbial suspension of approximately $1.5 \times 10^8$ c.f.u./ml [27]. 20 ml of agar media was poured into each petri plate these plates were then swabbed with a colony from inoculum of the test microorganisms and kept for adsorption for 15 min. Using sterile cork borer of 6 mm diameter, wells were bored into the seeded agar plates and these were loaded with 50 μl volume with concentration of 10 mg/ml of each compound reconstituted in dimethylsulphoxide (DMSO). All the plates were incubated at 37 °C for 24 h. Antibacterial activity of all the complexes was evaluated by measuring the diameter of zone of inhibition in mm. The medium with dimethylsulphoxide (DMSO) as solvent was used as a negative control whereas media with ciprofloxacin (standard antibiotic for gram positive) and gentamicin (standard antibiotic for gram negative) were used as positive control. The experiments were performed in triplicates.

#### 2.4.3. Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of microorganisms after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to antimicrobial agents and also to monitor the activity of new antimicrobial agents. The MIC of the macrocyclic complexes was tested against bacterial strains through a broth dilution method. In this method, the test concentrations of all the complexes were made from 2.5 to 0.01 mg/ml in the sterile wells of the micro-titer plates. In sterile microtitre plates (96-u-shaped wells) 50 μl of the sterile nutrient broth was poured in each well in three rows, then from fresh inoculums so formed ($10^8$ c.f.u./ml diluted with 100 μl Nutrient broth to have $10^6$ c.f.u./ml) 50 μl of the suspension was poured in each well in the first and third row, second row was again filled with 50 μl of nutrient broth, finally the drug sample 50 μl was added in the first row diluting uniformly from 2.5 to 0.01 mg/ml till the 8th well. All the microtitre plates were incubated at 37 °C for 18–24 h. MIC was expressed as the lowest dilution, which inhibited the growth of bacteria observed by lack of turbidity in the well.

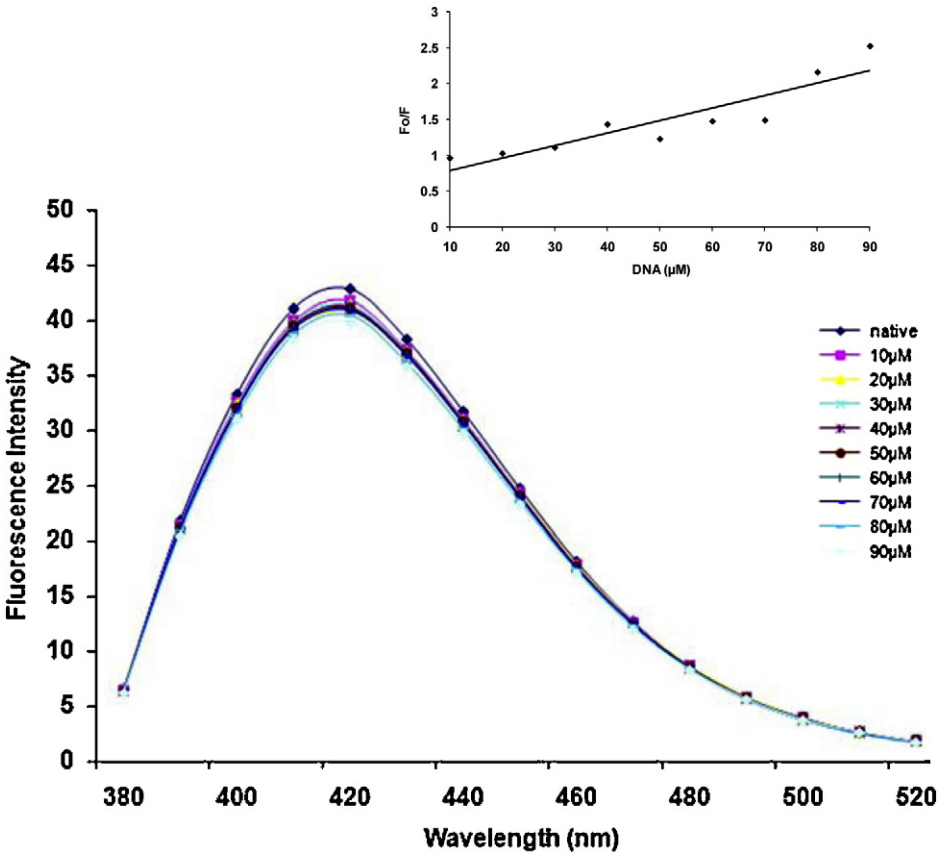



**Fig. 1.** Fluorescence emission spectrum of Co(II) complex in presence of increasing amount of CT-DNA. Inset shows the Stern–Volmer plot for the binding with CT-DNA.

### 2.5. DNA binding studies

#### 2.5.1. The fluorescence studies of ethidium bromide bound to DNA in the presence of metal complexes

The experiment was carried out at pH 7.0 in the buffer containing 50 mM NaCl and 5 mM Tris–HCl. DNA and ethidium bromide (EB) were dissolved in buffer at the concentrations of 3 and 1 μg/ml, respectively. The concentrations of the tested complex were 50 μM. EB displays very weak fluorescence in aqueous solution. However, in the presence of DNA, it exhibits intense fluorescence because of the intercalation to base pairs in DNA. All the complexes were added to EB bound with ct DNA and the intensity of fluorescence of EB was measured. Fluorescence spectra were recorded using excitation wavelength of 478 nm and the emission range set between 505 and 645 nm. Before examining the fluorescent properties of EB, it was checked that the tested compounds did not quench the EB fluorescence.

#### 2.5.2. Binding data analysis of the complexes

To elaborate the fluorescence mechanism the Stern–Volmer equation [28] was used for data analysis.

$$\frac{F_0}{F} = 1 + K_{SV}[Q]$$

where, $F$ and $F_0$ are the fluorescence intensity with and without the quencher (complex-DNA), $K_{SV}$ the Stern–Volmer quenching constant, and [$Q$] the concentration of the quencher. The $K_{SV}$ value of the complexes [$CoLCl_2$], [$NiLCl_2$], [$CuLCl_2$] and [$ZnLCl_2$] were calculated to be $1.74 \times 10^4$, $1.5 \times 10^5$, $3.83 \times 10^5$ and $3.6 \times 10^5\ M^{-1}$, respectively. A higher $K_{SV}$ value of Cu(II) complex suggest its stronger quenching ability than other complexes. This implicates the higher binding affinity of Cu(II) complex toward the DNA than other complexes.

The state of the quenching due to molecular interaction between luminophore and quencher can be purely static or purely dynamic. However, these two states are the extremes and in most cases the upward curvature of Stern–Volmer plot represents a combination of both static and dynamic quenching as stated above which occur simultaneously. The data plotted as emission intensity against quencher concentration (Figs. 1–4) gives an upward curve in our case as well, thus indicating the incidences of both static and dynamic quenching.

## 3. Results and discussion

The tetraaza Schiff base macrocyclic complexes of the type [$MLCl_2$] [M = Co(II), Ni(II), Cu(II), Zn(II)] have been synthesized by [2+2] condensation reaction between 3,4-diaminotoulene and benzil (Scheme 1). The purity of the complexes was checked by thin layer chromatography (TLC) run in 1:1 benzene–methanol. However, inspite of all possible efforts a single crystal suitable for X-ray crystallography could not be successfully isolated. All the complexes were stable at room temperature and soluble in DMSO. The molar conductance data of the $10^{-3}$ M solution of the complexes measured in DMSO indicated the non-electrolytic nature of all the complexes. The analytical data along with some physical properties of the complexes are summarized in Table 1. The formation of Schiff base complexes and bonding modes were inferred from positions of characteristic bands in FT-IR spectra and resonance signals in $^1H$ and $^{13}C$ NMR spectra. The overall geometry of all the complexes was deduced from the observed values of magnetic moment and the position of the bands in electronic spectra.

**Table 1**
Elemental analyses, color, yield, molar conductance, and melting point values of complexes.

| Complexes | Color | Yield (%) | Found (calc.) % | | | | | Molar conductivity ($ohm^{-1}$ $cm^2$ $mol^{-1}$)/m.p. (°C) |
|---|---|---|---|---|---|---|---|---|
| | | | M | Cl | C | H | N | |
| $C_{42}H_{32}N_4CoCl_2$ | Dark brown | 42 | 8.22 (8.15) | 9.62 (9.81) | 69.40 (69.81) | 4.22 (4.46) | 7.55 (7.75) | 30/>300 |
| $C_{42}H_{32}N_4NiCl_2$ | Brown | 48 | 8.25 (8.12) | 9.55 (9.81) | 69.60 (69.83) | 4.15 (4.46) | 7.48 (7.75) | 12/>300 |
| $C_{42}H_{32}N_4CuCl_2$ | Light green | 50 | 8.50 (8.73) | 9.52 (9.75) | 69.19 (69.37) | 4.20 (4.43) | 7.41 (7.70) | 33/>300 |
| $C_{42}H_{32}N_4ZnCl_2$ | Pale white | 51 | 8.75 (8.96) | 9.40 (9.72) | 69.08 (69.19) | 4.18 (4.42) | 7.36 (7.68) | 14/>300 |

### 3.1. IR spectra

The preliminary identification regarding formation of the complexes was obtained from the IR spectral findings (Table 2). The IR spectra of all the complexes do not show bands corresponding to free amino or carbonyl group rather a strong intensity band appeared in the region 1618–1622 $cm^{-1}$ characteristic of azomethine group $\nu$(C=N) [29]. The presence of medium intensity band in the region 440–460 $cm^{-1}$ assignable to $\nu$(M–N) vibration [30] confirms the coordination of azomethine nitrogen to metal ions. The bands characteristic of aromatic ring vibrations appeared in the1440–1447, 1026–1061, 700–770 $cm^{-1}$ regions in all the complexes. A weak absorption band in the region 2906–2942 $cm^{-1}$ may be assigned to $-CH_3$ stretching vibration. The coordination of the chloro group has been ascertained by bands in 255–290 $cm^{-1}$ region, which may be assigned to $\nu$(M–Cl) vibration [31].

### 3.2. $^1H$ and $^{13}C$ NMR spectra

The $^1H$ NMR spectrum gives some important information regarding the formation of proposed macrocyclic moiety (Fig. 5). The $^1H$ NMR spectrum of macrocyclic Zn(II) complex recorded in DMSO-$d_6$ shows a sharp signal at 2.08 ppm which may reasonably be assigned to the methyl protons ($-CH_3$; 6H) [32]. Multiplets observed in the region 7.23–7.96 ppm may be attributed to aromatic ring protons [33].

The $^{13}C$ NMR spectrum of the Zn(II) complex shows a number of sharp peaks corresponding to various carbon atoms in the proposed structure, resulting due to the non-equivalence of the various carbon atoms (Fig. 6). A sharp signal observed at 153 ppm may be assigned to azomethine carbon [34]. The resonance signals for aromatic carbons appeared in the 127–141 ppm region [35]. A sharp signal is observed at 21 ppm corresponding to methyl carbons [36].

### 3.3. Mass spectra

The mass spectra of macrocyclic compounds, showed molecular ion peaks (M+1) at $m/z$ 723, 723, 728 and 730 for [$C_{42}H_{32}N_4CoCl_2$], [$C_{42}H_{32}N_4NiCl_2$], [$C_{42}H_{32}N_4CuCl_2$] and [$C_{42}H_{32}N_4ZnCl_2$], respectively which are in good agreement with the respective molecular formulae. The mass spectrum of Cu(II) complex has been depicted in Fig. 7. The spectrum shows a molecular ion peak (M+1) at $m/z$

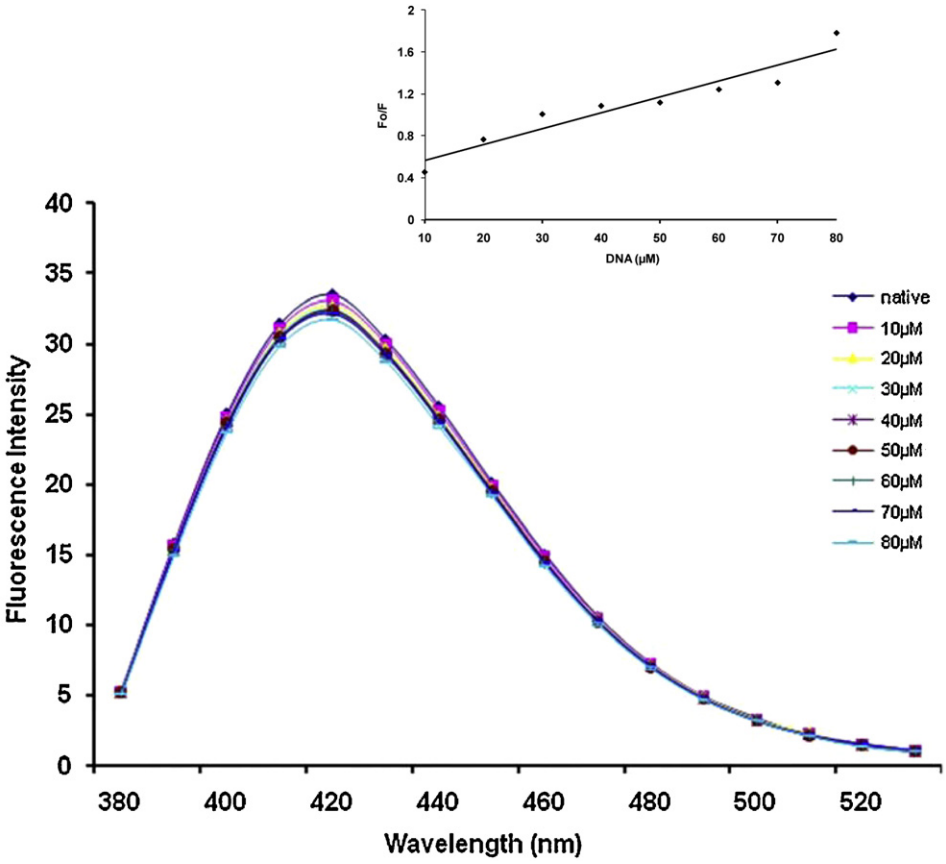



**Fig. 2.** Fluorescence emission spectrum of Ni(II) complex in presence of increasing amount of CT-DNA. Inset shows the Stern–Volmer plot for the binding with CT-DNA.

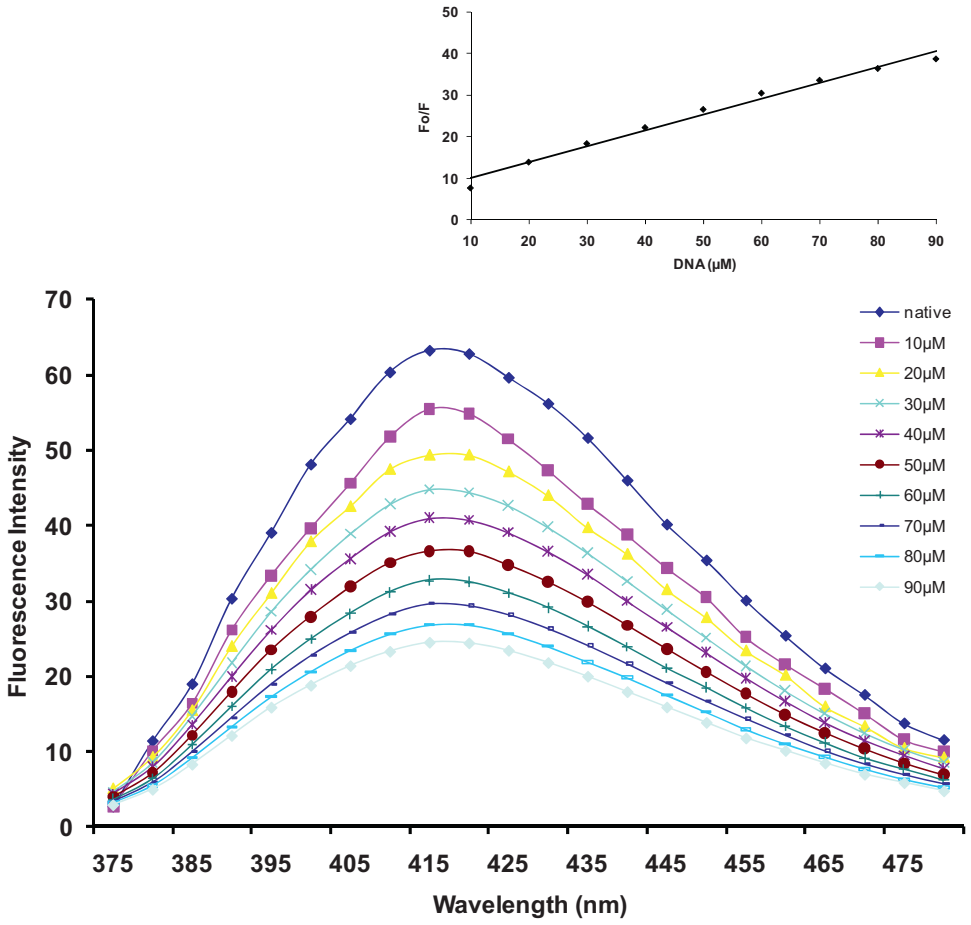



**Fig. 3.** Fluorescence emission spectrum of Cu(II) complex in presence of increasing amount of CT-DNA. Inset shows the Stern–Volmer plot for the binding with CT-DNA.

728, the fragment peaks observed at 712 and 658 are due to the cleavage of $CH_3$ and Cl, respectively.

### 3.4. EPR spectrum

The EPR spectral studies of Cu(II) complex provides information of the metal ion environment. The EPR spectrum of the Cu(II) complex was recorded in DMSO at liquid nitrogen temperature (LNT). The spectrum did not show any hyperfine splitting, and only a single signal was observed (Fig. 8). The Cu(II) complex exhibits the $g_{||}$ value of 2.12 and $g_{\perp}$ value of 2.03. The trend $g_{||} > g_{\perp} > 2.0023$ observed for the complex indicate that, the unpaired electron is localized in the $d_{x2-y2}$ orbital of the Cu(II) ion characteristic of the axial symmetry, leading to a tetragonally elongated structure of the Cu(II) complex [37]. The calculated $g_{av}$ value of 2.06 and its deviation from that of the free electron (2.0023) may be due to the covalence in M—L bonding which is supported by Kivelson and Neiman [38]. However, the calculated G parameter of 3 which is less than 4 indicates considerable exchange interaction present between the Cu(II) centers [39].

### 3.5. Electronic spectra and magnetic susceptibility data

The electronic spectra of the macrocyclic Co(II) complex exhibit three spin allowed bands at 8500, 14,260 and 21,500 $cm^{-1}$ corresponding to $^4T_{1g}(F) \rightarrow {}^4T_{1g}(F)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ transitions, respectively consistent with the octahedral geometry around the cobalt(II) ion which is further supported by a magnetic moment value of 4.49 B.M [40].

The electronic spectrum of Ni(II) complex exhibit three bands at 9000, 15,400 and 22,550 $cm^{-1}$ attributed to three spin allowed transitions *viz.* $^3A_2g(F) \rightarrow {}^3T_2g(F)$, $^3A_2g(F) \rightarrow {}^3T_1g(F)$ and $^3A_2g(F) \rightarrow {}^3T_1g(P)$, respectively corresponding to an octahedral geometry for the Ni(II) complex. The observed magnetic moment value of 3.12 B.M further complements the electronic spectral findings [40,41].

The electronic spectrum of the copper(II) complex show bands at 16,250 and 20,500 $cm^{-1}$ which may be ascribed to $^2B_{1g} \rightarrow {}^2Eg$ and $^2B_{1g} \rightarrow {}^2B_{2g}$ transitions, respectively corresponding to distorted octahedral geometry. Magnetic moment of 1.83 B.M. further confirms an octahedral geometry around the Cu(II) ion [40].

**Table 2**
IR vibrational frequencies ($cm^{-1}$) of the complexes.

| Complexes | ν(C=N) | ν(M—N) | ν(M—Cl) | ν(C—H) | Phenyl ring vibrations |
|---|---|---|---|---|---|
| $[CoLCl_2]$ | 1621 | 439 | 272 | 2925 | 1442, 1060, 776 |
| $[NiLCl_2]$ | 1620 | 436 | 255 | 2942 | 1447, 1062, 701 |
| $[CuLCl_2]$ | 1618 | 460 | 275 | 2919 | 1440, 1026, 770 |
| $[ZnLCl_2]$ | 1622 | 430 | 290 | 2906 | 1445, 1061, 700 |

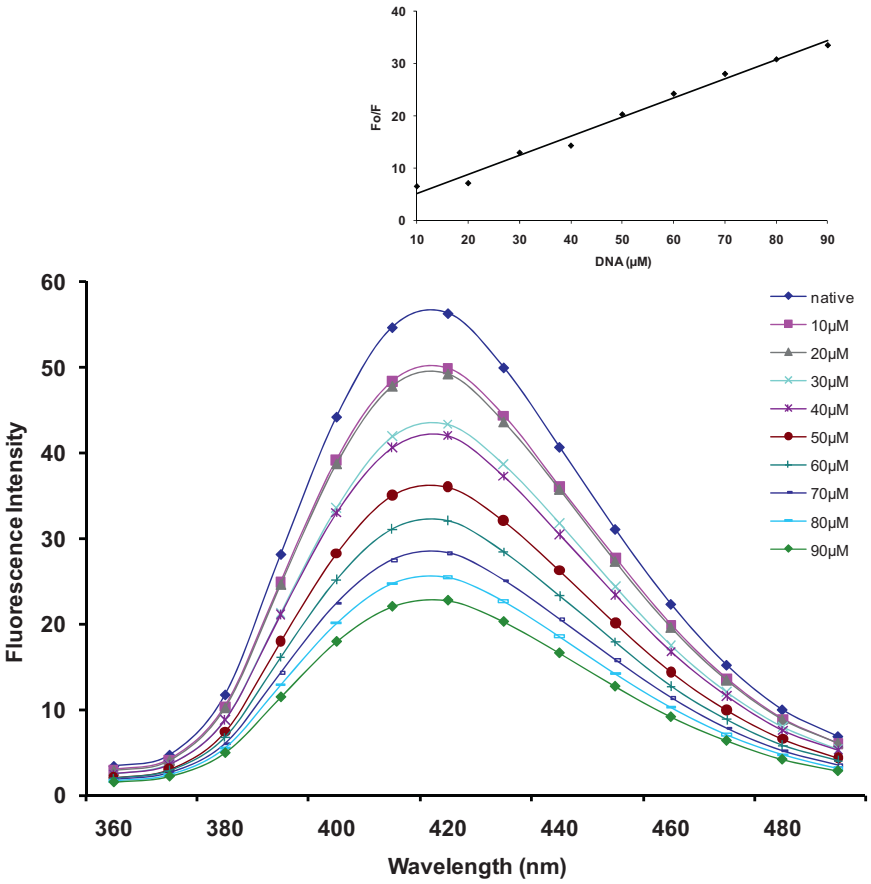



**Fig. 4.** Fluorescence emission spectrum of Zn(II) complex in presence of increasing amount of CT-DNA. Inset shows the Stern–Volmer plot for the binding with CT-DNA.

### 3.6. Antibacterial activity

Antibacterial activity of the synthesized complexes was studied against some bacterial strains *viz*. *S. aureus*, *S. mutans*, *S. pyogenes*, *S. epidermidis*, *B. cereus*, *C. xerosis*, *E. coli*, *K. pneuomoniae*, *P. vulgaris* and *P. aeruginosa*. Preliminary screening for all the complexes was performed at fixed concentration of 10 mg/ml. The results obtained were compared with standard antibiotics: ciprofloxacin (for gram-positive) and gentamicin (for gram-negative) bacterial strains. All the complexes were found to be active on both types of bacterial strains. On the basis of the data obtained for diameter of zone of inhibition Cu(II) and Ni(II) complexes were found to be very effective (Fig. 9a and b). In addition all the complexes were found to be effective at different dilutions based on the activity. The minimum inhibitory concentration of these complexes was determined by broth dilution method in which the effectiveness was observed at lower concentrations (Table 3). It was observed from the MIC values that Cu(II) complex was quite effective against *S. mutans* (0.156 mg/ml), *S. pyogenes* (0.312 mg/ml), *C. xerosis* (0.312 mg/ml), *K. pneuomoniae* (0.312 mg/ml), *E. coli*

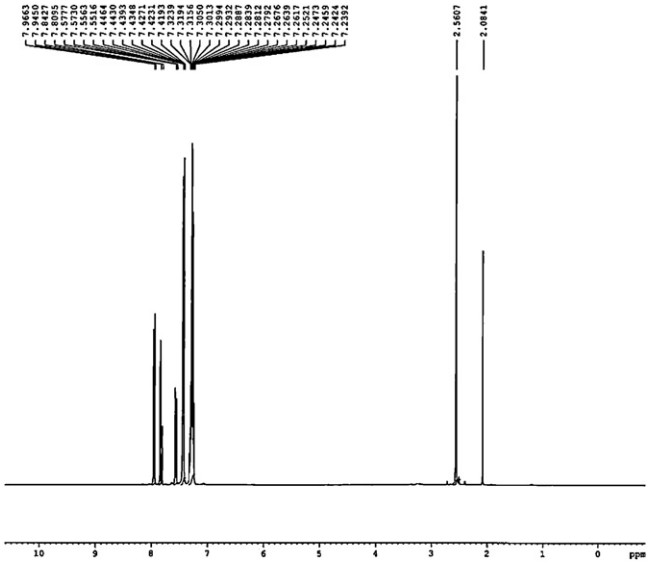



**Fig. 5.** $^{1}$H NMR spectrum of Zn(II) complex.

**Table 3**
Minimum inhibitory concentration (MIC in mg/ml) of the macrocyclic complexes.

| S.no | Bacterial Strains | Complexes | | | |
|---|---|---|---|---|---|
| | | $[CoLCl_2]$ | $[NiLCl_2]$ | $[CuLCl_2]$ | $[ZnLCl_2]$ |
| 1. | *S. aureus* | 0.312 | 0.625 | 2.500 | 1.250 |
| 2. | *S. mutans* | 0.625 | 0.312 | 0.156 | 2.500 |
| 3. | *S. epidermidis* | 1.250 | 0.625 | 2.500 | 1.250 |
| 4 | *S. pyrogenes* | 0.625 | 0.312 | 0.312 | 1.250 |
| 5. | *B. cereus* | 2.500 | 0.625 | 1.250 | 2.500 |
| 6. | *C. xerosis* | 1.250 | 1.250 | 0.312 | 1.250 |
| 7. | *E. coli* | 2.500 | 2.500 | 0.625 | 0.625 |
| 8. | *K. pneuomoniae* | 0.312 | 0.156 | 0.312 | 1.250 |
| 9. | *P. aeruginosa* | 2.500 | 1.250 | 1.250 | 0.625 |
| 10. | *P. vulgaris* | 1.250 | 0.625 | 0.625 | 1.250 |

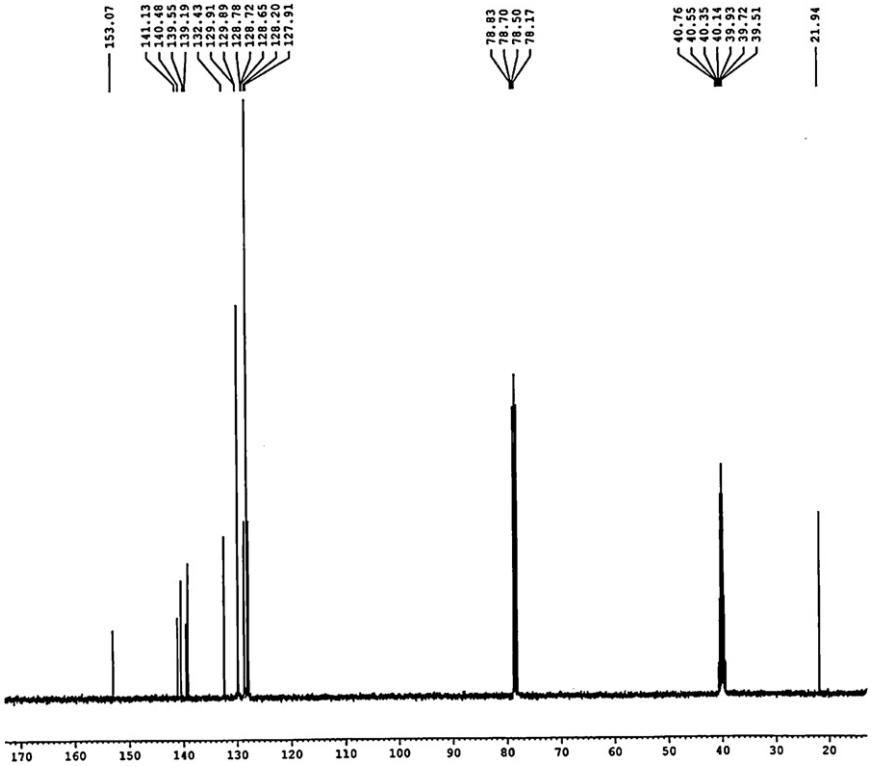



**Fig. 6.** $^{13}$C NMR spectrum of Zn(II) complex.

(0.625 mg/ml) and *P. vulgaris* (0.625 mg/ml). The MIC for Ni(II) complex was found to be 0.156 mg/ml for *K. pneuomoniae*, 0.312 mg/ml for *S. mutans* and *S. pyogenes* and 0.625 mg/ml for *S. aureus*, *S. epidermidis*, *B. cereus* and *P. vulgaris*. For Co(II) complex MIC was highest for *S. aureus* and *K. neuomoniae* (0.312 mg/ml) and then 0.625 mg/ml for *S. mutans* and *S. pyogenes*. The Zn(II) complex showed comparatively low activity against most of the bacterial strains.

It is concluded that all the synthesized macrocyclic complexes showed the antibacterial activity, but they were found to be more potent inhibitors against gram positive bacterial strains (Table 3). The Cu(II) complex showed comparatively more inhibition against *K. pneuomoniae* as compared to the commercial antibiotic (Fig. 9b).

The reason for high antimicrobial activity of copper complex can be explained in terms of the effect of copper metal ion on the normal cell process. The complexation reaction reduces the polarity of the metal ion by the partial sharing of metal ion positive charge with donor groups and electron delocalization over the chelate ring [42]. Thus, the lipophilic character of the central metal atom is enhanced which results in a higher capability to penetrate the microorganisms through the lipid layer of the cell membrane.

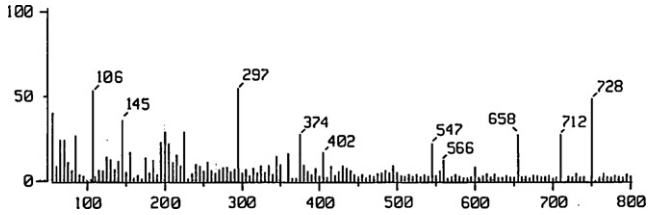



**Fig. 7.** FAB-mass spectrum of Cu(II) complex.

### 3.7. Fluorescence measurements

#### 3.7.1. DNA binding of the complexes

Fluorescence quenching is a useful method to monitor the molecular interactions of chemical and biological systems because of its high sensitivity [43,44]. Fluorescence intensity of a

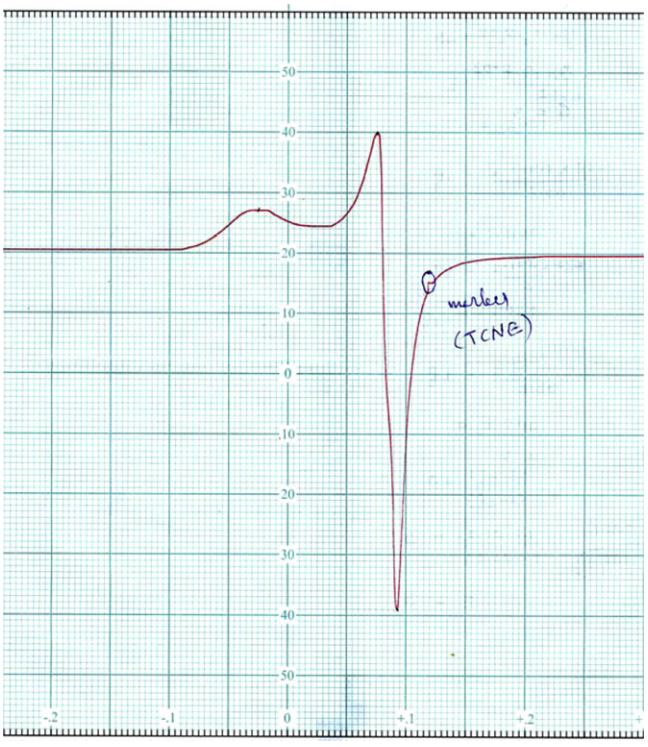



**Fig. 8.** EPR spectrum of Cu(II) complex.

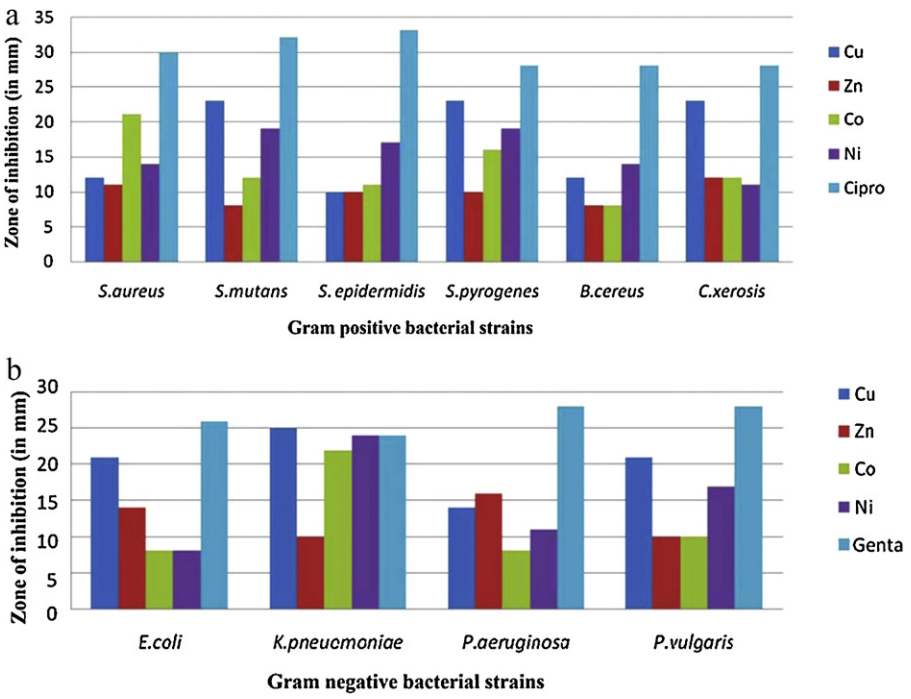



**Fig. 9.** (a) Comparison of zone of inhibition (in mm) of macrocyclic complexes with standard antibiotic against gram positive bacterial stains. Ciprofloxacin-standard antibiotic. (b) Comparison of zone of inhibition (in mm) of macrocyclic complexes with standard antibiotic against gram negative bacterial stains. Gentamicin-standard antibiotic.

compound can quench as a result of variety of molecular interactions such as excited state reactions, molecular rearrangements, energy transfer, ground state complex formation and collisional quenching. Dynamic quenching results from collision between fluorophore and quencher whereas static quenching is due to ground-state complex formation between fluorophore and quencher [45]. In the present study, we investigated the interactions of synthesized macrocyclic complexes with calf thymus DNA by fluorescence spectroscopy. Fluorescence quenching is usually classified as dynamic quenching and static quenching [46]. However, the characteristic Stern–Volmer plot of combined quenching (both static and dynamic) is an upward curvature. The binding of macrocyclic complexes with calf thymus DNA, was studied by monitoring the changes in the intrinsic fluorescence of different complexes at varying DNA concentrations. The representative fluorescence emission spectra of the complexes upon excitation at different wavelengths are given in Figs. 1–4. The spectra illustrate that excess of DNA caused a gradual decrease in the fluorescence emission intensity. The quenching of the fluorescence clearly

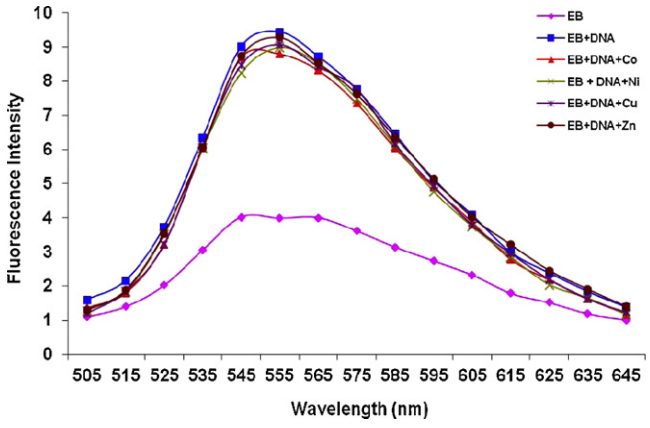
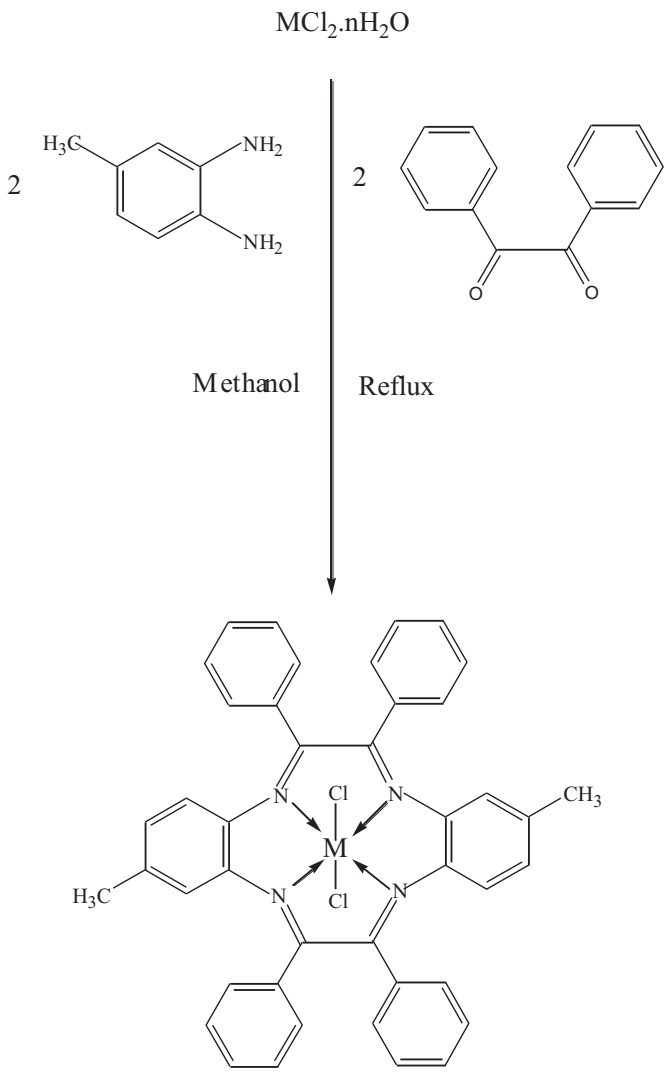



**Fig. 10.** Flourescence emission spectra of ethidium bromide bound to DNA in absence and presence of Co(II), Ni(II), Cu(II) and Zn(II) complexes.

indicates that the binding of DNA to the macrocyclic complexes changed the microenvironment of fluorophore residue. The spectra illustrate that excess of DNA led to more effective quenching of fluorophore molecule fluorescence (Fig. 10). There are three major mode of DNA interaction relevant to the metal complexes depending on the presence of charged atoms, hydrophobicity and structure of these complexes. As external binders the complexes with positive charges interact with the DNA backbone due to the electrostatic interaction with negatively charged phosphates. Groove binders interact with the DNA groove and hydrophobic interactions are usually important components of this binding process. Such a mode of interaction is also governed by geometric and steric factors such as non-planer structures and the presence of methyl groups that prevent intercalation. The third mode involves the insertion of a planar fused aromatic ring system between the DNA base pairs leading to intercalation [47–49]. Few studies on tetrazamacrocyclic complexes have proposed intercalation as a possible mode of DNA interaction [50,51]. In order to examine the possible mode of interaction of the complexes with DNA, ethidium bromide displacement assay has been performed. Ethidium bromide, a polycyclic aromatic dye, is the most widely used fluorescence probe for DNA structure. It binds to DNA by intercalation within the stacked bases [52]. It has been reported that the enhanced fluorescence of the EB-DNA complex can be quenched at least partially by the addition of a second molecule and this could be used to assess the relative affinity of the molecule for DNA intercalation [53]. The emission spectra of EB bound to DNA in the absence and presence of complexes is given in Fig. 10. The addition of these complexes to DNA being complexed with EB does not cause reduction in emission intensity, indicating that none of these complexes compete with EB in binding to DNA. The external interaction with DNA back bone was also ruled out as these complexes are non-cationic, lacking in a nucleophilic attracting centre. These aromatic complexes with methyl groups possibly interact with DNA within the grooves *via* stabilization through hydrophobic cohesion. This is presumably explained due to steric hindrance encountered by methyl groups, thus preventing the DNA base intercalation of these complexes.




M = [Co(II), Ni(II) n = 6, Cu(II) n = 2, Zn(II)]

**Scheme 1.** Synthesis and proposed structure of tetraazamacrocyclic complexes.

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