Asian Journal of Chemistry; Vol. 32, No. 12 (2020), 3012-3018

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2020.22874

Synthesis, Characterization of Metal Complexes of Schiff Base derived from Pyrrole-2-carbaldehyde and 4-Methoxy Aniline and their Biological Studies

A. CHARLES[1], R. KRISHNAVENI[2] and K. SIVARAJ[3,*]

[1]Research and Development Centre, Bharathiar University, Coimbatore-641046, India
[2]Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences, Thandalam-602105, India
[3]Department of Chemistry, Tamilavel Umamahesuwaranar Karanthai Arts College, Thanjavur-613002, India

*Corresponding author: E-mail: sivaa25@gmail.com

Received: 27 June 2020; *Accepted*: 24 August 2020; *Published online*: 7 December 2020; AJC-20132

The metal complexes of Fe(II), Co(II) and Ni(II) have been synthesized from *N*-((1*H*-pyrrol-2-yl)methylene)-4-methoxyaniline. The synthesized Schiff base and its metal complexes were structurally characterized based on IR, NMR, UV-vis spectroscopic techniques. The presence of coordinated water molecules were confirmed by thermal studies. All the synthesized compounds were screened for their antibacterial (*Escherichia coli*, *Bacillus substilis*, *Salmonella typhi*, *Staphylococcus epidermis*) and antifungal (*Aspergillus flavus*) and DNA cleavage activity.

Keywords: Antibacterial, Antifungal, DNA cleavage, Schiff base, Metal complexes, Pyrrole-2-carbaldehyde.

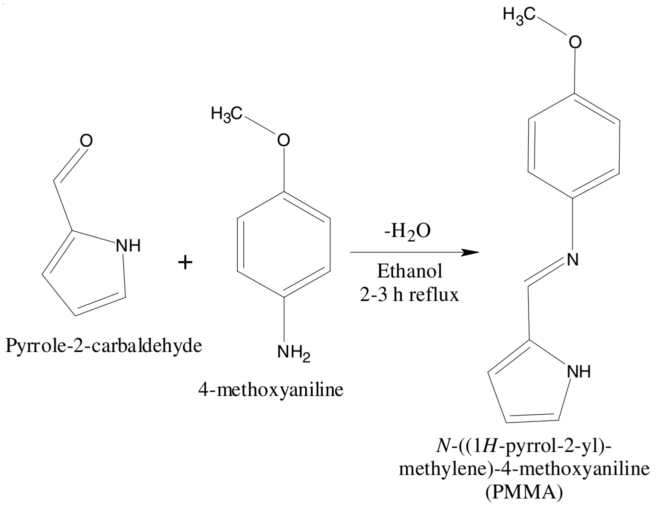
INTRODUCTION

Schiff bases are regarded as “privileged ligands” due to their capability to form metal complexes with a wide range of transition metal ions yielding stable and intensely coloured metal complexes [1]. New materials with interesting properties like specific sensors, magnetic and optical devices and molecular wires can be obtained by successful formulation of compounds with distinctly different metal ion binding sites within the same ligand [2]. Schiff base metal complexes attract considerable interest and an important role in the development of such new materials. The reason is attributed to the chemistry of chelate systems of Schiff bases, especially those with N_2O_2 or N_4 tetra-dentate ligands, which closely resemble metalloproteins [3]. Synchronized applications of a wide variety of Schiff bases with different metal complexes having such tetradentate donor atoms around the metal ion have been reported to catalyse reactions such as epoxidation, hydroformylation, hydroge-nation, electrochemical investigation and biological studies of proteins as well [4]. Amalgamation of Schiff bases with metallo-elements (transition metals) enhancing the stability, formation constants and enhancing the biological activity of synthesized Schiff base have also been reported. Such metal complexes are widely used as a model antibacterial agent due to their growth inhibiting activity towards bacteria and fungi [5]. Such heteronuclear Schiff base complexes have found tremendous applications in the field of bio-engineering [6]. Organoiron(III) complexes and copper(II) complexes are widely reported for their enhanced selectivity and enabled photo-toxicity [7]. In particular, the coordination chemistry of Schiff base complexes of carbaldehyde have attained much attention during the last decade [8]. Samarium(II) complexes of symmetrical N_4 tetradendate Schiff base ligands are found to be highly stable due to the introduction of pyrrole ring has been reported [9]. Based on these findings, this study aims to synthesize and characterize novel inorganic complexes obtained by the reaction of Fe(II), Co(II) and Ni(II) salts with *N*-((1*H*-pyrrol-2-yl)-methylene)-4-methoxyaniline. Additionally, antimicrobial and biological studies were also carried out. With the choice of the metals here, an effective photophysical investigation of the complexes is anticipated in future owing to their emitting characteristics [10].

EXPERIMENTAL

All the chemicals and reagents used were of analytical grade and procured from Sisco Research Laboratories, India. Metal salts were purchased from E. Merck (India) Ltd. and used as received. The solvent used were either spectroscopic pure or purified by the recommended methods. All the apparatus used during the experimental work were fitted with quick fit interchangeable standard ground joints. Melting points were measured using a capillary melting point apparatus. Shimadzu FT-IR spectrophotometer and JASCO UV-Vis spectrophotometer were used for IR and absorption recordings respectively. Emission studies were done using Perkin-Elmer spectrophotometer. Thermogravimetry measurements were done with TGA instrument with horizontal balance and furnace temperature was maintained at 1200 ºC with a heating rate of 5 to 20 ºC/min. ^{1}H NMR spectrometric measurements were done with Bruker EXT40178, 400 MHz NMR spectrometer using deuterated acetone as solvent. Mass spectral analysis were done using a Perkin-Elmer GC-MS mass spectrophotometer. A Vario (EL III) analyzer was used for elemental analysis.

Synthesis of Schiff base ligand: *N*-((1*H*-Pyrrol-2-yl)-methylene)-4-methoxyaniline (PMMA) was synthesized according to reported method [11] (**Scheme-I**). Condensation of pyrrole-2-carbaldehyde and 4-methoxy aniline was carried out in ethanol. The reaction mixture was refluxed for 3-4 h and allowed to cool. The products were filtered, washed and recrystallized from the same solvent and dried. The compound appeared yellowish brown in colour and had a glittering texture. The purity of the product was checked by TLC method, yield: 64%, m.p. 239 ºC, m.f. $C_{12}H_{12}N_2O$ and *m.w.* 200.24 (calcd). The consigned molecular formula and mass were arrived based on mass spectral analysis in which the molecular ion peaks, [M^+] appeared at *m/z* = 200.2031 with intensity 80% and [M+1] peak appeared at 201.2462 with intensity 10%, confirming the stoichiometry of the ligand.



Scheme-I: Synthesis of PMMA ligand

Synthesis of metal-Schiff base complexes: Fe(II), Co(II) and Ni(II) complexes with the Schiff base ligand were prepared by adding of equimolar solution of corresponding metal(II) salt and the ligand using alcohol as the solvent. The mixture was maintained under refluxing condition for 4-5 h. On cooling solid complexes were precipitated, filtered, washed with cold ethanol and dried in a vacuum desiccator over fused $CaCl_2$. The yield of the metal complexes was 56, 72 and 64%, respectively.

RESULTS AND DISCUSSION

The reaction of Schiff base ligand (PMMA) with Fe(II), Co(II) and Ni(II), ions in an equimolar ratio resulted in $[M(PMMA)_2(Cl)_2]$ complexes. There was a good agreement observed between the physical and analytical data with the proposed composition of Schiff base ligand and its metal(II) complexes. The newly synthesized complexes were found to be insoluble in water and organic solvents like DMSO and DMF, but was very stable in air. They all appeared coloured and amorphous in nature. The elemental analysis of ligand and its metal(II) complexes are given in Table-1.

IR studies: FTIR spectrum of the ligand was recorded to confirm the formation of the ligand moiety. The absence of any peak around 3200 cm^{-1} ($-NH_2$) indicated the absence of any residual starting material methoxy aniline in the compound [12]. The band due to (C=N) group, which was the link between the aldehyde and amine molecules appeared at 1616 cm^{-1}, thus establishing the confirmation of the formation of Schiff base ligand. The other important observations from IR spectrum were the peaks at 1029 and 1439 cm^{-1}, which were attributed to the ν(C-O-C) and ν(Ar-N), respectively.

Electronic spectroscopic studies: The absorption spectrum of the ligand synthesized was recorded which evidenced the successful formation of the ligand moiety through the characteristic maximum absorption at 321 nm which is attributed to π-π^* transition of the C=N link.

NMR characterization: The NMR recordings further confirmed the linkage between the starting materials and proved the preservence of the structural moiety of the proposed ligand. The ^{1}H NMR spectrum of the ligand (Fig. 1) showed the signals of the aromatic protons of aniline between δ 6.6 to 7.2 (double triplets, 6H), methoxy protons at δ 3.762 ppm (s, 3H), the new link C-C=N at δ 8.313 (s,1 H) and pyrrole N at δ 11.663 (s, 1H).

This was strongly supported by the 2D COSY NMR spectrum (Fig. 2) which shows no cross-coupling of this proton

TABLE-1
ELEMENTAL ANALYSIS OF THE PMMA LIGAND AND ITS METAL COMPLEXES WITH Fe(II), Co(II) AND Ni(II)

Compound	Colour	Yield (%)	m.w.	Elemental analysis (%): Found (calcd.)			
				M	C	H	N
*PMMA	Yellowish brown	64	200.24	–	71.98 (72.04)	6.04 (6.09)	13.99 (13.92)
$[Fe(PMMA)_2Cl_2]\cdot 4H_2O$	Reddish brown	56	527.23	10.59 (10.54)	54.68 (54.65)	4.59 (4.61)	10.63 (10.69)
$[Co(PMMA)_2Cl_2]\cdot 6H_2O$	Black	72	530.32	11.11 (11.06)	54.36 (54.39)	4.56 (4.59)	10.57 (10.59)
$[Ni(PMMA)_2Cl_2]\cdot 6H_2O$	Black	64	530.08	11.07 (11.09)	54.38 (54.40)	4.56 (4.54)	10.57 (10.54)

*PMMA refers to the ligand-($C_{12}H_{12}N_2O$).

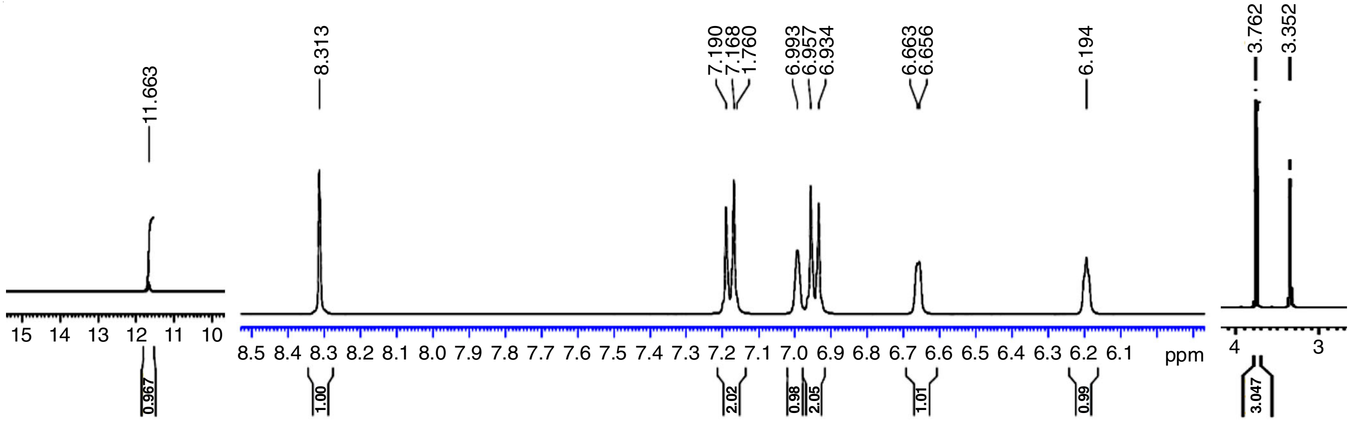


Fig. 1. ^{1}H NMR spectrum of ligand (PMMA). Insets show the signals corresponding to aromatic and methoxy protons

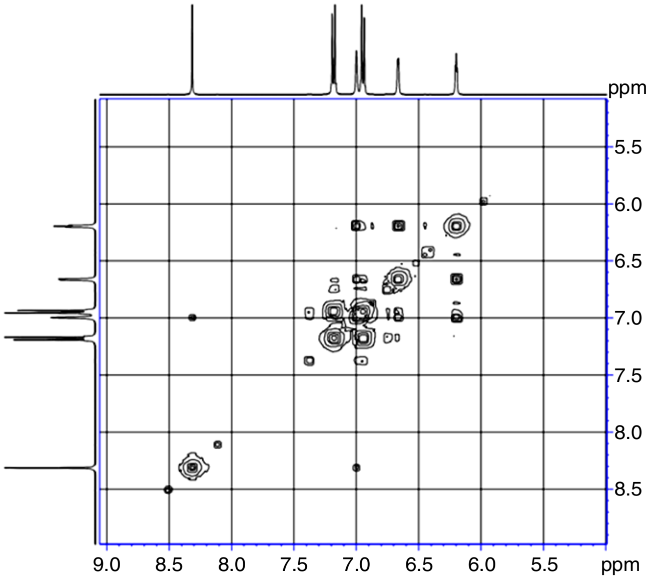


Fig. 2. 2D COSY NMR spectrum of ligand (PMMA)

at C1. The aromatic protons obtained as two triplets at 6.9 and 7.1 δ ppm are attributed to the cross coupling of the two protons at C2 and C3 positions of the benzene ring as established by the 2D NMR spectrum.

Thermal analysis: The physical and chemical stability of the ligand was also checked with DTA and TGA studies, respectively. The DTA curve of the ligand shows two endothermic peaks at 95.39 °C and 239.05 °C in the thermal analysis, which are indicative of dehydration reactions and melting of the compound respectively and no net chemical reaction occurs on heating the sample (Table-2). Also the TGA curve shows the thermal stability of the ligand shows a massive stability as a solid even at high temperatures (Fig. 3).

Electronic spectroscopic studies: The UV-vis spectrum of Fe(PMMA)$_2$ complex showed three characteristic absorption maxima as observed at 316, 398 and 645 nm. The peak at 316 nm is the signature peak of the PMMA ligand and the broader peaks at longer wavelengths are assigned to the n→π*, C-T transition and *d-d* transition of the metal, respectively.

The UV-vis absorption spectrum of Co(PMMA)$_2$ complex also showed a similar behaviour with the peaks occurring at 321, 439 and 651 nm. While in the case of Fe(PMMA)$_2$ Schiff base complex, here again the peaks were assigned to the π→π* of the ligand, n→π* charge transfer and *d-d* transitions of the metal based complex respectively. Similar peaks corresponding to these transitions were observed at 324,358 and 692 nm for Ni(PMMA)$_2$ complex. The broad band observed at longer wavelengths for all the three metal complexes are in accor-dance with crystal field stabilization energy requirements for an octahedral geometry of metal(II) complexes [13] *viz.*, 15100, 14534, 14450 cm^{-1}. Thus, octahedral geometry for the complexes was first designated by the electronic spectra of the metal complexes which were further supported by present thermal analyses.

TABLE-2
TGA DATA OF M (PMMA)$_2$ SCHIFF BASE COMPLEXES

Compound	Dissociation stages	Decomposition temperature (°C)	Mass loss (%)		Assignment
			Found	Calculated	
[Fe(PMMA)$_2$Cl$_2$]·4H$_2$O	Stage I	100-250	15.97	16.52	$4H_2O$, OCH_3
	Stage II	250-540	15.99	16.84	C_7H_8O
	Stage III	540-780	13.92	13.96	C_6H_6O
	Stage IV	Above 780	52.68	52.73	Residue
[Co(PMMA)$_2$Cl$_2$]·6H$_2$O	Stage I	80-300	24.3	25.68	$6H_2O$, C_2H_5OH, CH_3
	Stage II	300-780	13.86	14.24	C_6H_6O
	Stage III	Above 780	59.93	60.11	Residue
[Ni(PMMA)$_2$Cl$_2$]·6H$_2$O	Stage I	60-140	4.56	4.69	OCH_3
	Stage II	140-400	27.13	27.95	$C_{12}H_{12}N_2$
	Stage III	400-780	16.07	16.2	C_6H_7NO
	Stage IV	Above 780	52.59	51.14	Residue

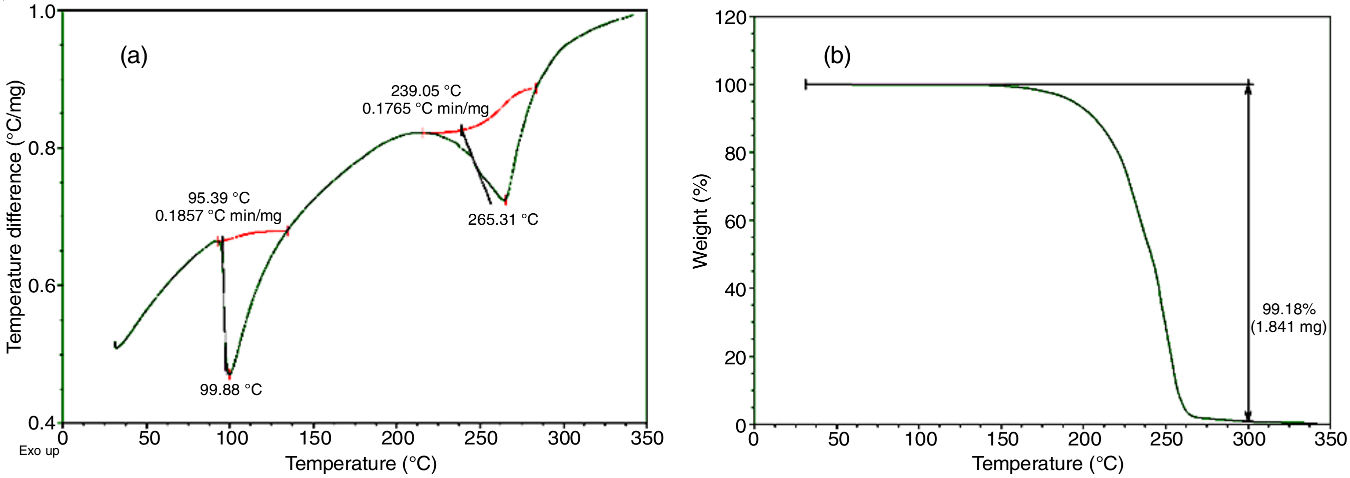


Fig. 3. Thermal analysis of ligand (PMMA) (a) DTA and (b) TGA spectrum

IR studies: The IR absorption spectra of the Schiff base metal complexes were recorded in order to confirm the functional groups. The peak positions of the prominent functional groups and links were compared with the peaks of the ligand and the shifts were also compared as collated in Table-3.

The above comparison emphasizes a negative shift of the peaks and their change in intensities. This observation is attributed to the difference in binding modes of the complexes with reference to the free ligand. Hence, the phenomenon chelation is predicted in the metal complexes which also may involve the coordination of donor atoms of the ligand with the metal ions.

NMR studies: The ^{1}H NMR spectrum recorded for the Fe(PMMA)$_2$ complex (Fig. 4a) revealed the retainment of all the signals of the ligand except a slight shift in their chemical shift positions. This is attributed to change in the geometry of the structure which is expected to complexation with a metal. The peaks are assigned as methoxy protons at δ 3.824 ppm (s, 3H), aromatic protons between δ 6.6 and 7.4 (m, 6H), Ar-CH=N at δ 8.55 (s, 1H) and pyrrole N-H at δ 9.5 (s, 1H) ppm.

Similar behaviour was also observed with Co(PMMA)$_2$ complex (Fig. 4b). The peak assignments were methoxy protons at δ 3.83 (s, 3H), aromatic protons between δ 6.27 and 7.6 ppm (m, 6H), Ar-CH=N at δ 8.597 (s, 1H) and pyrrole N-H at δ 9.569 (s, 1H) ppm. The ^{1}H NMR spectrum of Ni(PMMA)$_2$ is as shown in Fig. 4c. The peaks obtained were assigned as methoxy protons at δ 3.833 (s, 3H), aromatic protons between δ 6.32 and 7.45 ppm (m, 6H), Ar-CH=N at δ 8.601 (s, 1H) and pyrrole N-H at δ 9.55 (s, 1H) ppm.

Antibacterial activity: The *in vitro* antimicrobial activity of the synthesized Schiff base ligand PMMA and its metal(II) complexes were assayed with bacterial pathogens namely *Escherichia coli* MTCC 433, *Bacillus substilis* MTCC 441, *Salmonella typhi* MTCC 3224 and *Staphylococcus epidermis* ATCC 12228. The procedure followed for carrying out the biological studies as described earlier [14]. Mueller-Hinton agar media were used for antibacterial studies. The pure dehydrated Mueller-Hilton agar (38 g) dissolved in 1000mL of distilled water. The pure cultures of the bacterial strains *E. coli*, *B. substilis*, *S. typhi* and *S. epidermis* were subcultured by inoculating in the nutrient broth and were incubated at 37 ºC for about 12 h [15]. The above media was used to prepare the agar plates and wells were dug with a sterile metallic cork borer 6 mm in diameter. Then 0.1 mL of fresh inoculum (containing around 1-2 × 10^6 CFU/mL as per McFarland standards) was spread onto the surface of sterile Mueller Hinton agar plates using a sterilized spreader. Then discs impregnated with crude extract of the complexes (1 mg/disc) and solvent controls were placed on plates with the help of a sterilized forceps and the plates were incubated aerobically at 37 ºC. Similarly a negative solvent control (DMSO) disc were placed on each pathogen inoculated plates. The entire microbial assay was carried out under strict aseptic conditions. The zone of inhibition (mm) of the different metals was examined after 12-18 h. The experiments were done in triplicate and the average values were calculated for antibacterial activity.

As illustrated, the ligand by itself does not show any antibacterial activity. But the metal complexes of PMMA were found to show optimal zones of inhibition. Though the Fe(II) complex of the ligand was found active only against *E. coli* bacteria, the Co(II) complex showed positive results against all the species tested (Table-4). The activity of the metal complexes as good antibacterial agents can be explained based on the chelation theory [16]. The antibacterial action is

TABLE-3
IR FREQUENCIES OF THE SCHIFF BASE (PMMA) AND ITS METAL COMPLEXES

Compound	ν(C=N)	ν(C–O–C)	ν(Ar–N)	ν(*para* sub.arene)	ν(M–N)
PMMA	1616	1029	1439	829	_
[Fe(PMMA)$_2$Cl$_2$]·6H$_2$O	1597	1028	1465	823	543
[Co(PMMA)$_2$Cl$_2$]·6H2O	1602	1026	1504	827	527
[Ni(PMMA)$_2$Cl$_2$]·6H$_2$O	1597	1026	1506	827	545

TABLE-4
ANTIBACTERIAL ACTIVITY SCHIFF BASE (PMMA) AND ITS METAL COMPLEXES AGAINST HUMAN PATHOGENIC BACTERIA

Pathogens	$[Fe(PMMA)_2]Cl_2·4H_2O$	$[Co(PMMA)_2]Cl_2·6H_2O$	$[Ni(PMMA)_2]Cl_2·6H_2O$	PMMA
E. coli	0.6	0.9	1.1	–
Bacillus subtilis	–	3.0	–	–
Staphylococcus epidermis	–	2.5	–	–
Salmonella typhi	–	2.0	–	–

Values are (Mean ± S.D); Zone of Inhibition in mm

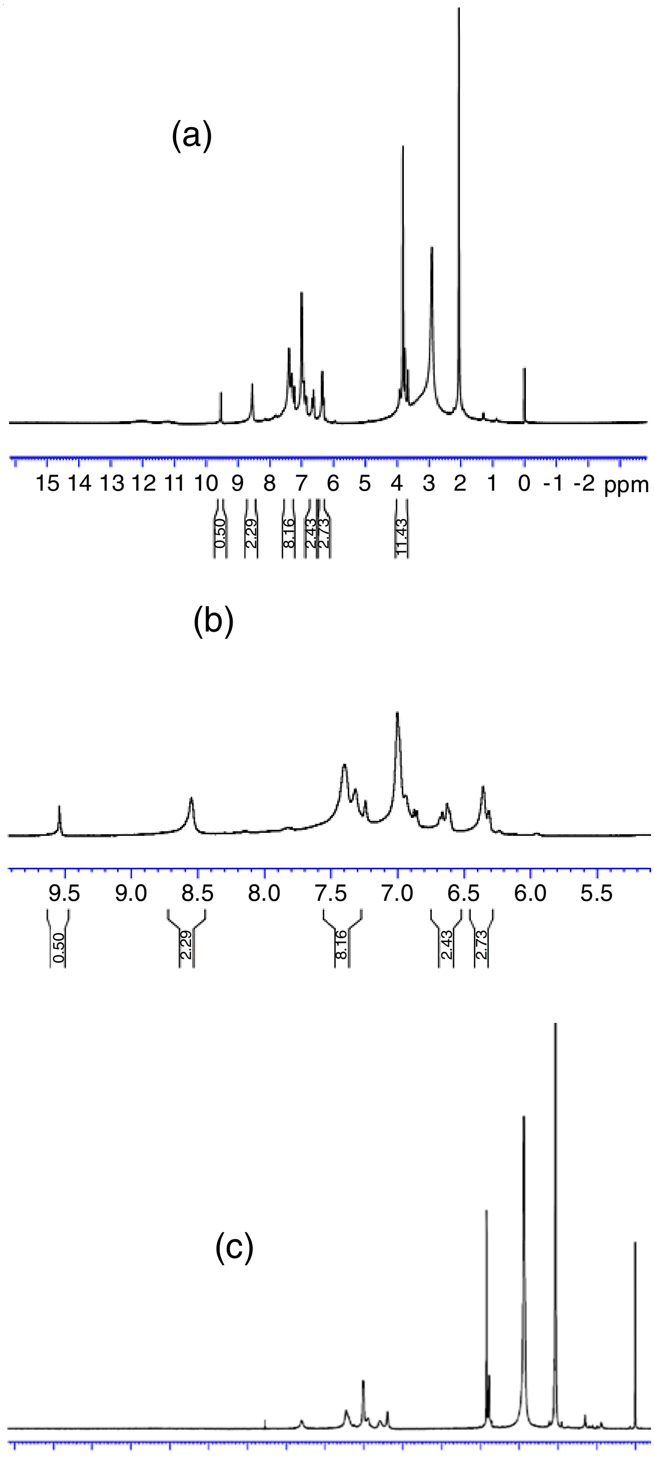


Fig. 4. ¹H NMR spectrum of (a) $Fe(PMMA)_2$ (b) $Co(PMMA)_2$ (c) $Ni(PMMA)_2$ Schiff base complexes

attributed to the principle that the positive charge of the metal is shared with the donor atoms of the ligand. This kind of sharing becomes possible due to the chelate structure formed between the ligand having a C=N bond and a metal possessing *d*-electrons which results in an overall delocalization of π-electrons over the entire chelate [17]. Such an electron delocalization eventually enhances the lipophilic nature of the metal chelate and favours its permeation through the lipid layer of the bacterial membranes hence acting as promising bactericides. The studies revealed that Fe(II) complexes are more active as antifungal agents than Co(II) and Ni(II) complexes.

Antifungal activity: For the synthesized PMMA ligand and its metal(II) complexes, minimum inhibitory concentration was determined in nutrient agar plate by micro dilution method, procedures followed being in accordance to the National Committee for Clinical Laboratory Standards [18]. The standardized suspension of test organisms (0.1 mL, 10^6 cfu/mL) and synthesized Schiff base ligand (PMMA) and its metal(II) complexes in different dilutions were taken with *Aspergillus flavus* as positive control for bacterial and fungal strains, respectively. The bacterial tubes were incubated at 37 ºC for 18 h and fungal tubes were incubated at 32 ºC for 48 h.

Among the synthesized metal(II) complexes, it is found that Co(II) complex are more active in antibacterial activities while Fe(II) complex in antifungal activities (Table-5). The difference in antimicrobial functions of the individual metal complexes with respect to various biological samples may be attributed to the difference in solubility, environmental conditions, nature of the species, *etc*.

TABLE-5
ASSAY OF ANTIFUNGAL ACTIVITY (*Aspergillus flavus*)

Sample	Zone of inhibition (mm in diameter)
Schiff base (PMMA)	–
$[Fe(PMMA)_2]Cl_2·4H_2O$	16
$[Co(PMMA)_2]Cl_2·2H_2O$	–
$[Ni(PMMA)_2]Cl_2·2H_2O$	–
Amphotericin B (100 Units/Discs) (Std.)	14
Control	–

DNA cleavage activity: The method of electrophoresis in a gel medium is used to carry out the study. The DNA cleavage of super coiled pBR322 DNA promoted by the synthesized metal(II) complexes was analyzed using agarose gel electrophoresis method [19,20]. The interaction of supercoiled plasmid DNA (pBR322 DNA) with the newly synthesized ligand PMMA and its metal(II) complexes are shown in Fig. 5. Ethidium bromide was used as the stock solution. The forms I, II, III

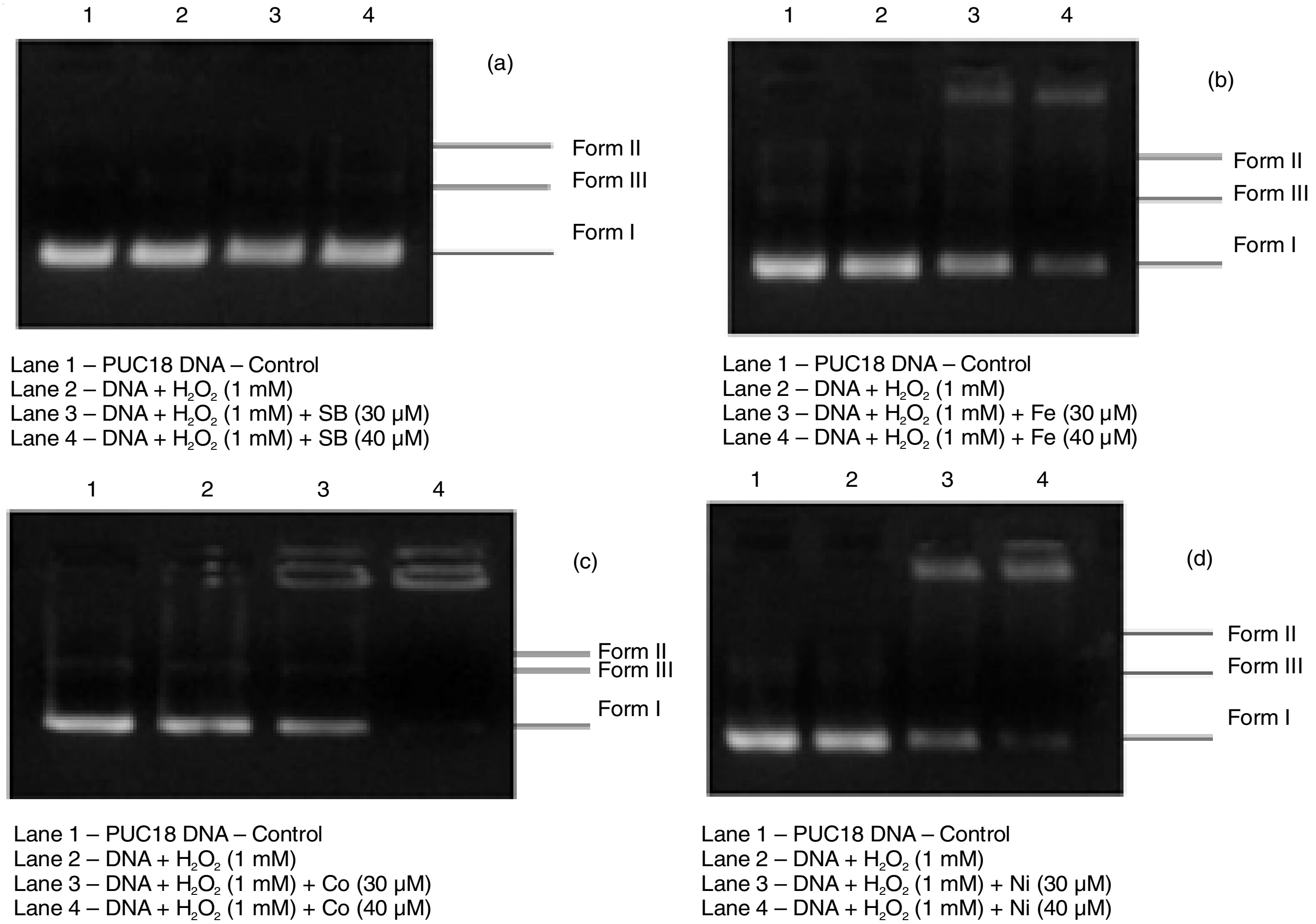


Fig. 5. DNA cleavage on plasmid pBR322: (a) PMMA (b) $Fe(PMMA)_2$ (c) $Co(PMMA)_2$ (d) $Ni(PMMA)_2$ complex

observed in the photoactivated image reveals the cleavage of the pBR322 DNA supercoiled strand. This is observed in the presence of PMMA ligand and all the synthesized metal(II) complexes. This indicates that the ligand and its metal(II) complexes have acted on DNA because of a difference in molecular weight between the control and the treated DNA samples. This reveals the influence of the newly synthesized complexes as good pathogenic microorganism inhibitor.

Conclusion

The present work focuses on the synthesis, characterization and biological studies of Schiff base complexes of Fe(II), Co(II) and Ni(II) the synthesized complexes were characterized by UV-vis, IR, NMR, mass spectroscopic techniques. Thermal analyses were also carried out to ensure the composition of the metal complexes. The ligand and its metal complexes were tested for their antimicrobial functions and found to be successful. The antimicrobial function of the complexes against pathogenic organisms (*Escherichia coli, Bacillus substilis*, *Salmonella typhi*, *Staphylococcus* and *Aspergillus flavus*) by measuring the zones of inhibition indicated the enhanced antimicrobial activity of the complexes over the controls used. A comparison of antimicrobial sensitivity of the complexes revealed that Fe(II) complex was found to be more active as an antifungal whereas Co(II) complex was more active as an antibacterial material. The complexes also showed an optimal efficiency for DNA cleavage function. Hence, the complexes studies are expected to have a profound scope towards anticancer studies.

CONFLICT OF INTEREST

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

REFERENCES

1. S. Chandra, S. Parmar and Y. Kumar, *Bioinorg Chem. Appl.*, **2009**, 851316 (2009); https://doi.org/10.1155/2009/851316
2. H.M. Parekh and M.N. Patel, *Russ. J. Coord. Chem.*, **32**, 431 (2006); https://doi.org/10.1134/S1070328406060066
3. Z.H.A. El-Wahab and M.R. El-Sarrag, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **60**, 271 (2004); https://doi.org/10.1016/S1386-1425(03)00216-6
4. F. Firdaus, K. Fatma, M. Azam and M. Shakir, *J. Coord. Chem.*, **63**, 3956 (2010); https://doi.org/10.1080/00958972.2010.526707
5. H. Keypour, M. Shayesteh, M. Rezaeivala, F. Chalabian, Y. Elerman and O. Buyukgungor, *J. Mol. Struct.*, **1032**, 62 (2013); https://doi.org/10.1016/j.molstruc.2012.07.056
6. E. Abele, *Main Group Met. Chem.*, **28**, 45 (2005); https://doi.org/10.1515/MGMC.2005.28.2.45

7. U. Basu, I. Pant, A. Hussain, P. Kondaiah and A.R. Chakravarty, *Inorg. Chem.*, **54**, 3748 (2015); https://doi.org/10.1021/ic5027625
8. C.M. Alvarez, R. Garcia-Rodriguez and D. Miguel, *Dalton Trans.*, **32**, 3546 (2007); https://doi.org/10.1039/b702418e
9. C.D. Berube, S. Gambarotta, G.P.A. Yap and P.G. Cozzi, *Organometallics*, **22**, 434 (2003); https://doi.org/10.1021/om020773r
10. T. Kawamoto, M. Nishiwaki, Y. Tsunekawa, K. Nozaki and T. Konno, *Inorg. Chem.*, **47**, 3095 (2008); https://doi.org/10.1021/ic7020758
11. S. Chandra and P. Ballabh, *Int. J. Pharm. Sci. Res.*, **4**, 2393 (2013); https://doi.org/10.13040/IJPSR.0975-8232.4(6).2393-99
12. R.B. Xu, N. Zhang, H.Y. Zhou, S.P. Yang, Y.Y. Li, D.H. Shi, W.X. Ma and X.X. Xu, *J. Chem. Crystallogr.*, **42**, 928 (2012); https://doi.org/10.1007/s10870-012-0338-1
13. B. Lakshmi, K.N. Shivananda, G.A. Prakash, K.R.K. Rama and K.N. Mahendra, *Bull. Korean Chem. Soc.*, **32**, 1613 (2011); https://doi.org/10.5012/bkcs.2011.32.5.1613
14. A.W. Bauer, W.M.M. Kirby, J.C. Sherris and M. Turck, *Am. J. Clin. Pathol.*, **45(4_ts)**, 493 (1966); https://doi.org/10.1093/ajcp/45.4_ts.493
15. N.G. Yernale and M.B. Hire Mathada, *Bioinorg. Chem. Appl.*, **2014**, 314963 (2014); https://doi.org/10.1155/2014/314963
16. A.K. Sharma and S. Chandra, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **78**, 337 (2011); https://doi.org/10.1016/j.saa.2010.10.017
17. Z.H. Chohan, M. Arif, M.A. Akhtar and C.T. Supuran, *Bioinorg. Chem. Appl.*, **2006**, 83131 (2006); https://doi.org/10.1155/BCA/2006/83131
18. R.L. Londonkar, U. Madire Kattegouga, K. Shivsharanappa and J.V. Hanchinalmath, *J. Pharm. Res.*, **6**, 280 (2013); https://doi.org/10.1016/j.jopr.2013.02.010
19. NCCLS (National Committee for Clinical Laboratory Standards), Performance Standards for Antimicrobial Disc Susceptibility Test, Approved Standard M2-A6, NCCLS, Wayne, PA, USA, edn 6 (1997).
20. A. Sitlani, E.C. Long, A.M. Pyle and J.K. Barton, *J. Am. Chem. Soc.*, **114**, 2303 (1992); https://doi.org/10.1021/ja00033a003