



Fe(III), Co(II) & Cu(II) Complexes of N,N'-Bis[2-salicylideneamino]ethane-1,2-diamine: Preparation, characterisation and in vitro antibacterial activity

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Salicylaldehyde derived hexadentate Schiff base N,N'-Bis[2-salicylideneamino]ethane-1,2-diamine (TTS) and its complexes with Fe(III), Co(II) and Cu(II) metal ions have been prepared and characterised by elemental analysis, UV-visible, IR, ^1H NMR, EPR spectral analysis, mass spectrometry and XRD method. In vitro antibacterial activity of the Schiff base and its metal complexes against Gram positive bacterial strains *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative bacterial strains *Enterobacter*, *Escherichia coli* has revealed that the metal complexes are more active to the bacterial strains as compared to that of the Schiff base. The Co-TTS has the highest antibacterial activity against *Escherichia coli*, while the Fe-TTS complex is highly active against *Enterobacter*, *Staphylococcus aureus* and *Bacillus subtilis*.

Keywords: Hexadentate Schiff base (TTS), Fe(III), Co(II), Cu(II)-TTS complexes, In vitro antibacterial activity

Schiff bases have drawn attention in both chemistry and biology for selective and specific complexation with metal ions^{1,2}. Schiff base transition metal complexes have been of great interest in co-ordination chemistry for many years due to their facile synthesis³ and wide applications in the development of catalysts and enzyme catalytic reactions, pharmaceuticals, and material chemistry⁴⁻⁶. These complexes are used in diverse fields such as for the treatment of cancer⁷ and as antibacterial⁸⁻¹⁰, antiviral¹¹, fungicidal¹², and antitumor agents^{13,14}. A comprehensive literature survey indicates that among the bio-essential metals, iron, cobalt and copper complexes of Schiff base are considered as promising biologically active agents. Recently, several copper and cobalt complexes of Schiff base have been reported as potential anticancer and chemotherapeutic agents and some copper complexes have been established to be active both in vitro and in vivo¹⁵⁻¹⁷. Schiff bases are used for the synthesis of ionophores and these ionophores are used in separation of metal ions by extraction and liquid membrane transport methods¹⁸⁻²³. There are numerous examples of the pentadentate and hexadentate Schiff base metal complexes derived from the reaction of metal salts with salicylaldehyde and polyamines²⁴⁻²⁶.

In this piece of work, hexadentate Schiff base (TTS) and its complexes with Fe(III), Co(II) and Cu(II) metal ions have been synthesized,

characterised and screened for their in vitro antibacterial activity against Gram positive bacterial strains *Staphylococcus aureus*, *Bacillus subtilis*, and Gram negative bacterial strains *Enterobacter*, *Escherichia coli*.

Materials and Methods

Chemicals

Salicylaldehyde and triethylenetetramine (TETA) were purchased from Loba Chemie and S.D. Fine, respectively. Metal salts FeCl_3 , $\text{Co}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ and $\text{Cu}(\text{CH}_3\text{COO})_2$ were obtained from CDH. Dimethylsulphoxide (DMSO), ethanol and methanol were obtained from Merck and used without further purification.

Instruments

Melting point was determined by capillary melting point apparatus. IR spectra were recorded using Shimadzu 8400 FT-IR spectrometer in the range of $4000\text{-}600\text{ cm}^{-1}$ at Central Analytical Laboratories, Indore. ^1H NMR spectra were recorded on Bruker Avance II 400 NMR spectrometer using tetramethylsilane (TMS) as an internal standard at Sophisticated Analytical Instrumentation Facility (SAIF), Punjab University, Chandigarh. Elemental analysis (CHN) was carried out using Eurovector EA 3000 Elemental Analyzer and mass spectra were

recorded on positive ESI mode using Waters UPLC-TQD Mass Spectrometer at SAIF, Lucknow UP. The X-ray diffraction (XRD) pattern of Fe-TTS was recorded on Bruker d8 advance X-ray diffractometer using $\text{CuK}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$), 40 kV, 40 MA, $2\theta/\theta$ scanning mode at UGC-DAE Consortium for Scientific Research, Indore. EPR spectra were recorded on JES-FA200 at SAIF, IIT Madras, Chennai.

Preparation of Schiff base (TTS)

The Schiff base (TTS) was synthesized by the reaction of salicylaldehyde with TETA in 2:1 molar ratio²⁷. The solution of salicylaldehyde (10.48 mL, 0.10 mol) in ethanol (25 mL) was refluxed with solution of TETA (7.5 mL, 0.05 mol) in ethanol (25 mL) in the presence of 1 mL acetic acid at 80 °C for 4 h in a round bottom flask. After cooling the solution in the ice bath, yellow crystals obtained, which were filtered, washed with hexane followed with water and recrystallised with ethanol (Scheme 1). Yield: 78%. Melting point: 110 °C. Solubility: soluble in methanol, chloroform, DMF, DMSO and hot ethanol. Elemental analysis: for $\text{C}_{20}\text{H}_{26}\text{N}_4\text{O}_2$ calculated (%): C 67.8, H, 7.4; N, 15.8, found (%): C 72.9, H 10.1, N 18.0. IR (cm^{-1}): 3550 (N-H), 3380 (O-H), 2923, 2850 (C-H), 1631 (C=N). ^1H NMR (CDCl_3 , ppm): 8.34 (s, 2H, CH=N), 6.80-7.33 (m, 8H, Ar-H), 2.59-3.73 (m, 12H, CH_2 -N), 1.23 (m, 2H, -NH-). ESI + MS (m/z) for $\text{C}_{20}\text{H}_{26}\text{N}_4\text{O}_2$: calculated 354.45, found 355.

Preparation of Metal complexes

The metal complexes (Fig. 1) were prepared from the reaction of the metal salts FeCl_3 , $\text{Co}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$

and $\text{Cu}(\text{CH}_3\text{COO})_2$ with the salicylaldehyde and TETA in mole ratio 1:2:1 in methanol.

Preparation of Fe(III)-TTS complex

Salicylaldehyde (1 mL, 0.010 mol) was mixed with TETA (0.75 mL, 0.005 mol) in methanol (50 mL) in a round bottom flask. The solution turns yellow immediately. After 5 min, solution of FeCl_3 (1.35 g, 0.005 mol) in methanol (30 mL) was added drop wise to the Schiff base suspension. The reaction mixture was refluxed for 2 h, filtered and kept at room temperature for slow evaporation of the solvent. Dark blue crystals were obtained²⁸⁻³⁰. Yield: 51.99%. Melting point: 165 °C. Solubility: Soluble in water and DMSO. Elemental analysis: for $\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_2\text{Fe}$, calculated (%): C 58.9, H 5.9, N 13.7; found (%): C 56.3, H 5.4, N 15.3. IR (cm^{-1}): 3475, 3407, 3324, 3166 (C-H), 1627 (C=N). ESI + MS (m/z) for ($\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_2\text{Fe}$): calculated 408.27, found 408.

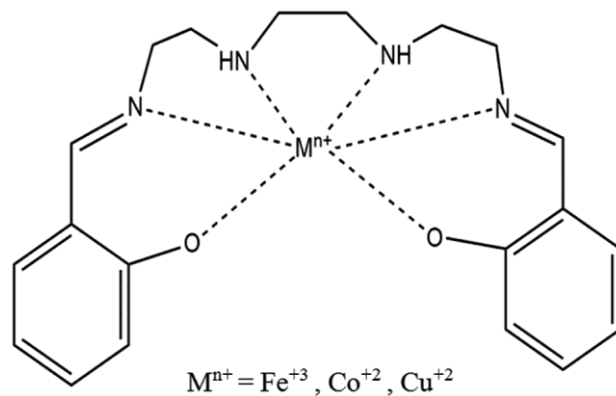
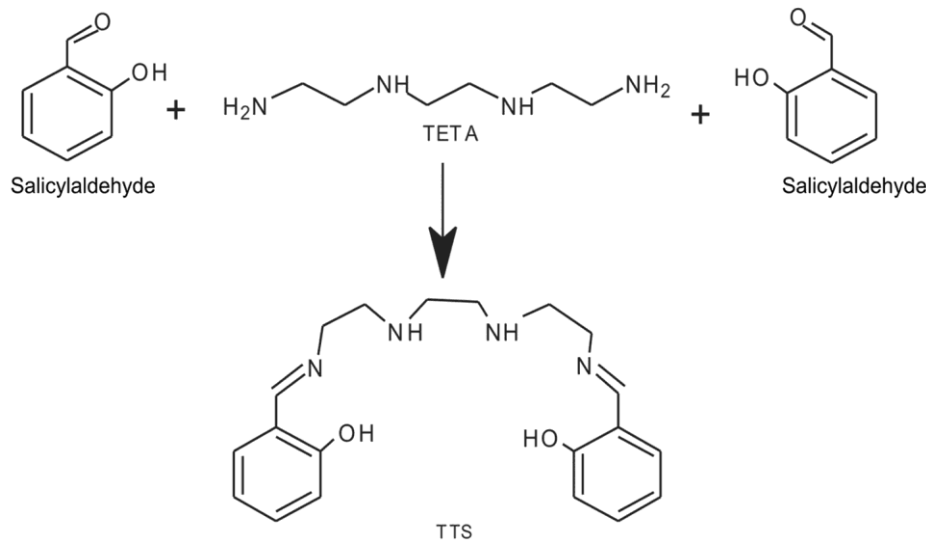


Fig. 1 — Structure of Metal complex of Schiff base



Scheme 1 — Synthesis of Schiff base (TTS)

Preparation of Co(II)-TTS complex

Salicylaldehyde (1 mL, 0.010 mol) was mixed with $\text{Co}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ (1.185 g, 0.005 mol) in methanol (60 mL) in a round bottom flask and stirred for 5 min. To this, solution of TETA (0.75 mL, 0.005 mol) in methanol (40 mL) was added drop wise and refluxed for 2 h. Black precipitates obtained, which were filtered, washed with methanol and dried in air^{31, 32}. Yield: 46.02%. Melting point: 218 °C. Solubility: Soluble in DMSO. Elemental analysis: for $\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_2\text{Co}$, calculated (%): C 58.3, H 5.9, N 13.6; found (%): C 50.9, H 7.3, N 19.2. IR (cm^{-1}): 3387, 3166, 2934 (C-H), 1636 (C=N). ESI + MS (m/z) for ($\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_2\text{Co}$): calculated 411.36, found 411.

Preparation of Cu(II)-TTS complex

Salicylaldehyde (1 mL, 0.010 mol) was mixed with anhydrous $\text{Cu}(\text{CH}_3\text{COO})_2$ (1.0 g, 0.005 mol) at 80 °C in methanol (50 mL) in a round bottom flask and stirred for 5 min. To this, solution of TETA (0.75 mL, 0.005 mol) in methanol (40 mL) was added drop wise and stirred at room temperature for 1 h. Brown crystals obtained, which were filtered, washed with methanol and dried in air^{24, 30, 33, 34}. Yield: 21.66%. Melting point: greater than 300 °C. Solubility: soluble in DMSO. Elemental analysis : for $\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_2\text{Cu}$, calculated (%): C 57.7, H 5.8, N 13.5; found (%): C 46.4, H 6.5, N 11.8. IR (cm^{-1}): 3490, 3050 (C-H), 1625 (C=N). ESI + MS (m/z) for ($\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_2\text{Cu}$): calculated 415.97, found 416.08.

In vitro antibacterial activity

Antibacterial activity of Schiff base and its metal complexes in DMSO was tested in triplicate against Gram positive bacterial strains *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative bacterial strains *Enterobacter*, *Escherichia coli* by the Agar well diffusion method. The wells of 6 mm center were dug in the media with the help of a sterile metallic borer. Recommended volume 50 μL of the test sample of different concentrations 10, 30, 50 mg/mL was introduced in the respective wells. The plates were incubated immediately at 37 °C for 24 h. Activity was

determined by measuring the diameter of zones showing complete inhibition (mm). Two standard drugs (antibiotics), Ampicillin (Zycillin-500) and Amoxicillin (MOX-500) were also screened against these bacterial strains for comparison. To examine the effect of DMSO on biological growth, separate studies were conducted with DMSO which showed no activity against these bacterial strains^{35,36}.

Results and Discussion

Characterization of complexes

Physical properties of Schiff base and its metal complexes are reported in Table 1. The FT-IR spectra of Schiff base and its metal complexes are shown in Supplementary Data, Figs S1-S4. The characteristic band at 1631 cm^{-1} for azomethine group (C=N) of Schiff base in Fig. S1 confirms the formation of Schiff base by condensation of keto group of salicylaldehyde and amino group of TETA while in Figs S2-S4, that band is shifted at 1627, 1636 and 1625 cm^{-1} for Fe(III), Co(II) and Cu(II) complexes, respectively, suggesting the involvement of nitrogen atom of azomethine group to coordinate metal ions. The absorption band at 3380 cm^{-1} is assigned for phenolic group (-OH) in Schiff base and disappeared in metal complexes suggesting the deprotonation of oxygen of phenolic group and chelation of deprotonated oxygen to metal ions²⁷.

The ^1H NMR spectrum of Schiff base (Supplementary Data, Fig. S5) shows a characteristic signal in the region 10-13 ppm (hump) for phenolic group (-OH), a peak at 8.34 ppm for azomethine group (C=N) and a complex multiplet in the region 6.80-7.33 ppm for aromatic protons, confirm the formation of Schiff base by condensation reaction of salicylaldehyde and TETA.

ESI mass spectra of Schiff base and its metal complexes support the proposed structures and composition (Fig. 1, Scheme 2 and Table 1). The mass spectra of Schiff base, Fe-TTS, Co-TTS and Cu-TTS (Supplementary Data, Fig. S6-S9) shows molecular ion peak at m/z 355 (calculated 354.45), 408 (calculated 408.27), 411 (calculated 411.36) and

Table 1 — Physical properties of Schiff base (TTS) and its metal complexes

Synthesized Compound	Colour	Stoichiometry	% Yield	Melting point (°C)	Molecular Formula	Molecular Weight	
						Calculated	Found
TTS	Yellow	-	78.00	110	$\text{C}_{20}\text{H}_{26}\text{N}_4\text{O}_2$	354.45	355
Fe-TTS	Dark blue	1:1	51.99	165	$\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_2\text{Fe}$	408.27	408
Co-TTS	Black	1:1	46.02	218	$\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_2\text{Co}$	411.36	411
Cu-TTS	Brown	1:1	21.66	>300	$\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_2\text{Cu}$	415.97	416.08

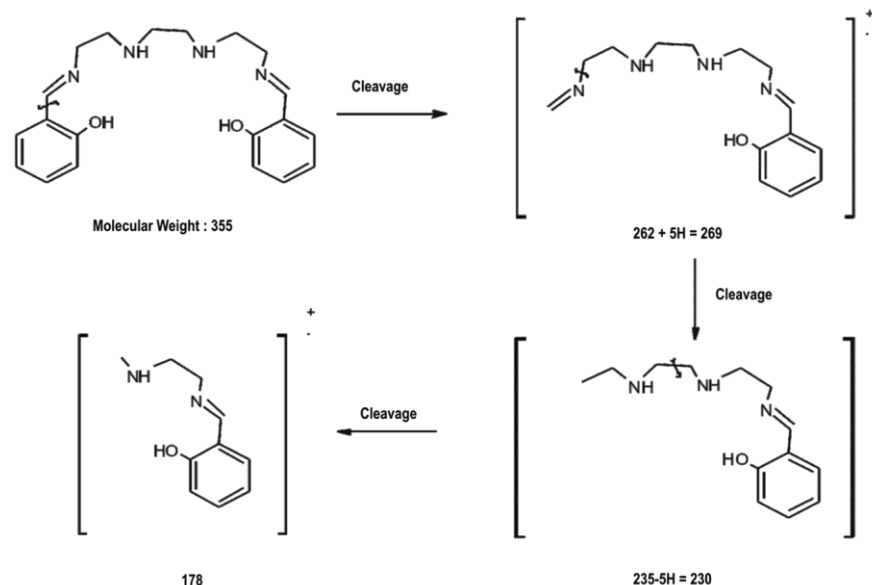
416.08 (calculated 415.97) corresponding to the molecular composition $C_{20}H_{26}N_4O_2$, $C_{20}H_{24}N_4O_2Fe$, $C_{20}H_{24}N_4O_2Co$ and $C_{20}H_{24}N_4O_2Cu$, respectively. The results show 1:1 ratio of metal to Schiff base in complexes.

The UV-visible spectra of Schiff base and metal complexes were recorded in methanol. The Schiff base exhibited intense bands in 200-400 nm region, it shows band at 254 nm assigned to $\pi-\pi^*$ electronic transition of phenyl ring, another band at 315 nm and 398 nm assigned to $\pi-\pi^*$ and $n-\pi^*$ electronic transition for imine (C=N) group, respectively. However in the Fe(III), Co(II) and Cu(II) complexes, the shift in $\pi-\pi^*$ transition band of phenyl ring and imine group was observed along with extra absorption band at 495, 572 and 574 nm with disappearance of band at 398 nm due to the coordination of the azomethine nitrogen atom (nitrogen lone pair of electron donation), corresponding to d-d transition, confirm the co-ordination of metal ion to Schiff base (Table 2 and Supplementary Data, Fig. S10)³⁷⁻³⁹.

The XRD pattern shown in Fig. 2 has been compared with the standard powder diffraction card

of JCPDS iron file no. 70-1517, five peaks at 2θ value of 14.7719, 21.6466, 29.7143, 34.7895 and 45.1825 ° in the experimental diffractogram have been identified and corresponding h k l values (4 1 0), (1 2 1), (3 3 1), (5 1 2) and (3 4 2) planes are labeled. The XRD study confirmed that the prepared Fe-TTS has monoclinic crystal lattice.

The average crystalline particle size 'D' of Fe-TTS has been estimated from the Fig. 2 using Debye-Scherrer formula, $D = 0.9\lambda/\beta\cos\theta$, where λ is wavelength of the X-rays used for diffraction and β is full width at half maximum (FWHM) of a peak. To estimate FWHM, each of the five peaks was fitted with a Gaussian function. The FWHM of the fitted Gaussian curve is taken as FWHM of the peak. This could be done by OriginPro 8.5 software, five values of the 'D' have been obtained from the data (Supplementary Data, Table S1) corresponding to the selected peaks. The average crystalline particle size of Fe-TTS is found to be 136.41 nm. The values of the interplanar spacing 'd' between the atoms have been calculated using Bragg's law. The average interplanar distance is found to be 3.5 Å^{40, 41}.



Scheme 2 — Mass analysis of Schiff base (TTS)

Table 2 — Characteristic electronic transition of Schiff base (TTS) and its metal complexes

Sample	Characteristic electronic transition (nm)			
	$\pi-\pi^*$ (Phenyl ring)	$\pi-\pi^*$ (Imine group)	$n-\pi^*$ (Imine group)	Transition due to M-L Coordination
TTS	201, 254	315	398	-
Fe-TTS	209, 231	336	-	495
Co-TTS	209, 259	339	-	572
Cu-TTS	207, 266	341	-	574

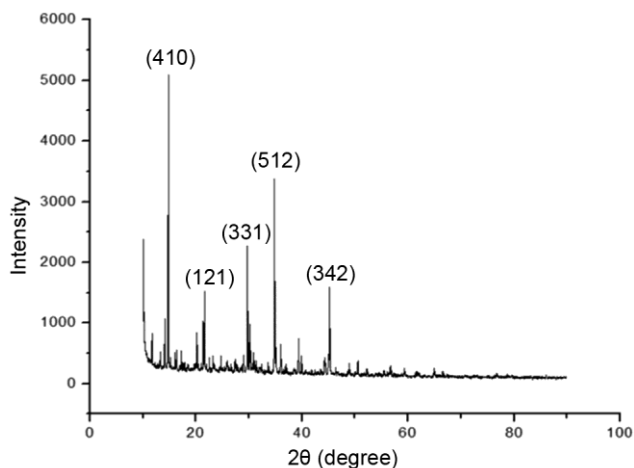


Fig. 2 — XRD plot of Fe-TTS

The EPR spectra of Fe-TTS, Co-TTS and Cu-TTS complexes were recorded at 77 K (Fig. 3). The g_{avg} values for Fe-TTS, Co-TTS and Cu-TTS complexes are 2.12, 2.15 and 2.12, respectively, which indicate distorted octahedral geometry around Fe(III), Co(II) and Cu(II) ions in these complexes. Three anisotropic signals are observed for Fe-TTS complex and four anisotropic signals are observed for Co-TTS and Cu-TTS complexes. From the observed g -values of these complexes, it is clear that $g_{\parallel} > g_{\perp}$ which indicates that the unpaired electron is predominantly present in the $d_{x^2-y^2}$ orbital and is the characteristic feature for the axial symmetry. The observed data showed that the g_{\parallel} values are greater than 2.0023 in complexes and hence the metal-ligand bondings in these complexes are essentially covalent. The average “ g ” value for overall distortion is calculated using the equation: $g_{\text{avg}} = 1/3(g_{\parallel} + 2g_{\perp})^{42,43}$.

In vitro antibacterial activity

The measured zone of inhibition against the growth of various bacterial strains is listed in Table 3 & 4 and presented in Fig. 4 and in Supplementary Data (Figs S11-S14). It is found that metal complexes have higher antibacterial activity as compared to that of Schiff base. Such increased activity of metal complexes can be explained on the basis of Overton's concept of cell permeability. According to this concept, the lipid membrane that surrounds the cell favours the passage of only lipid soluble material due to which lipid solubility is considered to be an important factor that control antibacterial activity. After complexation, the polarity of metal ion will be reduced to a greater extent due to the overlap of ligand orbital and partial sharing of positive charge of

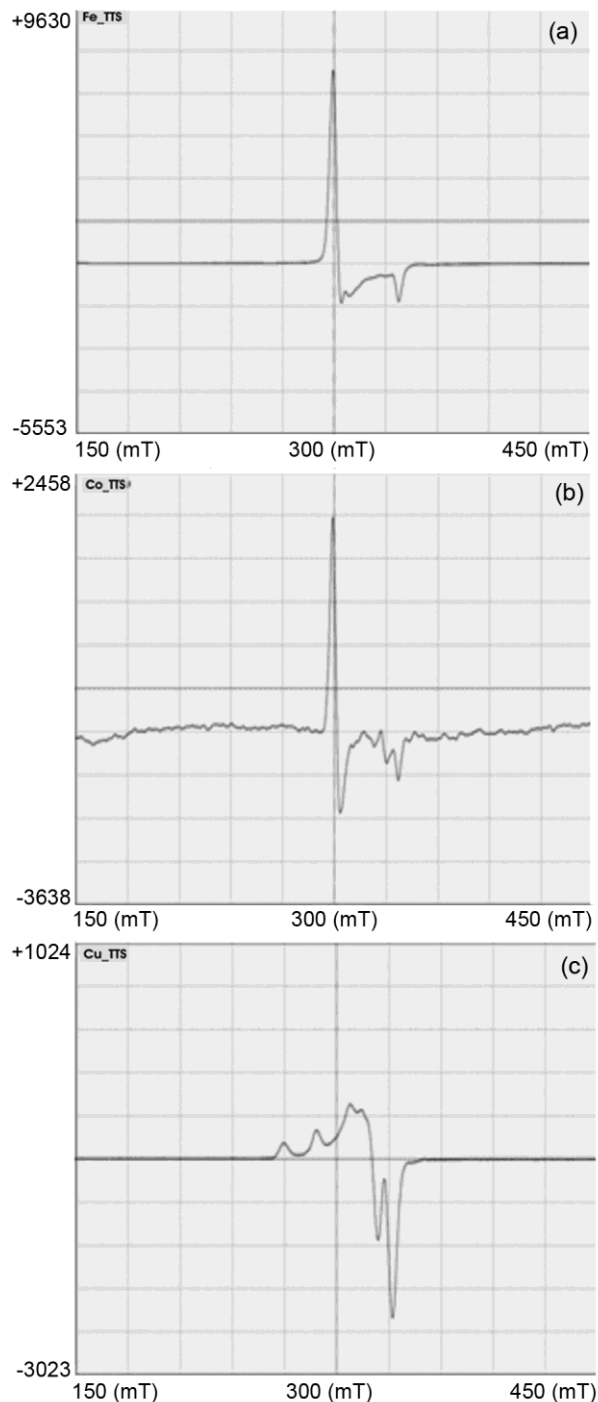


Fig. 3 — EPR spectra of Fe-TTS (a), Co-TTS (b) and Cu-TTS (c)

metal ion with donor groups. Further, it increases the π - electron delocalization over the whole ring and enhances the lipophilicity of the complex. As shown in Fig. 1, metal ion after complexation with schiff base form pseudocyclic structure which enhances lipophilicity, hence the penetration of the complexes into lipid membrane increases and blocks the metal

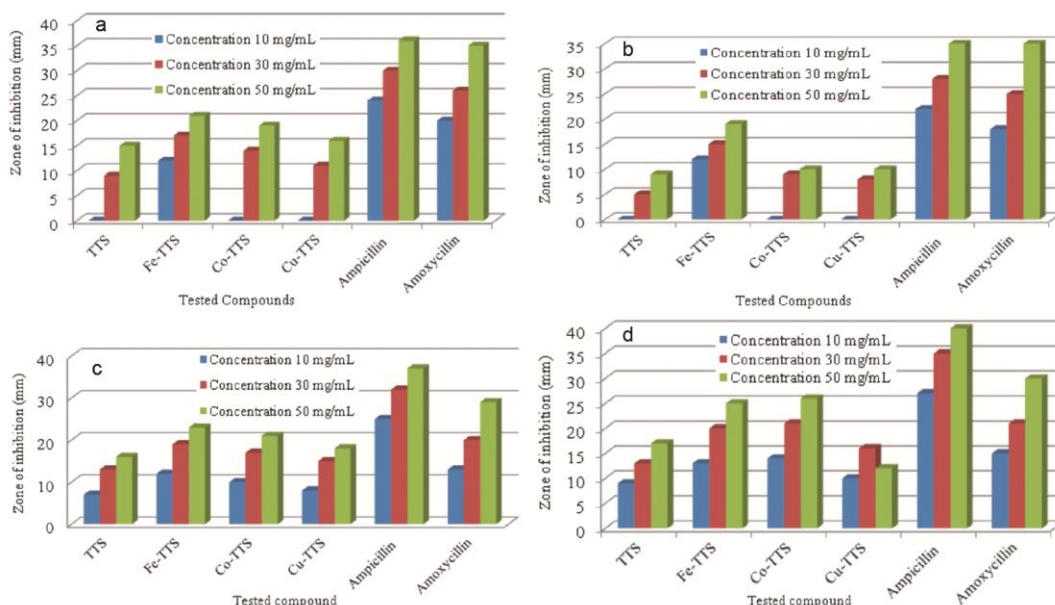


Fig. 4 — Antibacterial activity of synthesized compounds against (a) *Staphylococcus aureus*, (b) *Bacillus subtilis*, (c) *Enterobacter* and (d) *Escherichia coli*

binding sites on enzyme of microorganism. These metal complexes also disturb the respiration process of the cell and block the synthesis of proteins, which restricts further growth of the organism. The variation in the activity of different complexes against different organisms depend either on the impermeability of the cells of the microbes or difference in ribosomes of microbial cells⁴⁴⁻⁴⁸. However, compared with the standard drug (antibiotics) Ampicillin (Zycillin-500) and Amoxycillin (MOX-500) the activity exerted by Schiff base and metal complexes are found to be less.

Further it was observed that Schiff base and metal complexes are more active towards Gram negative bacterial strains compared to Gram positive bacterial strains, which depends on the composition of bacterial cell wall, as Gram negative bacteria have lipopolysaccharide layer in its cell wall, it may cause easy passage of Schiff base and metal complexes into the cell which restrict bacterial growth⁴⁹⁻⁵¹.

It is clear from Table 3 that the antibacterial activity of synthesized complexes against bacterial strains increases with increase in concentration however the Fe-TTS complex shows activity even at low concentration (10 mg/mL) as Fe-TTS complex is highly soluble in solvent as compare to other complexes. Co-TTS shows highest activity against *Escherichia coli*, Cu-TTS has least antibacterial activity against all bacterial strains while Fe-TTS is highly active against *Enterobacter*, *Staphylococcus aureus* and *Bacillus subtilis*.

Table 4 — Antibacterial activity of standards (antibiotics) Ampicillin (Zycillin-500) and Amoxycillin (MOX-500)

Bacteria	Zone of inhibition (mm)					
	Ampicillin (Zycillin-500)			Amoxycillin (MOX-500)		
Dilution (mg/mL)	10	30	50	10	30	50
<i>Enterobacter</i>	25	32	37	13	20	29
<i>Escherichia coli</i>	27	35	40	15	21	30
<i>Staphylococcus aureus</i>	24	30	36	20	26	35
<i>Bacillus subtilis</i>	22	28	35	18	25	35

Conclusions

Hexadentate Schiff base and its Fe(III), Co(II) and Cu(II) complexes have been prepared and characterized using different characterization techniques. The characterization studies revealed that the Schiff base (TTS) co-ordinate to the metal ion in 1:1 ratio through the phenolic oxygen and nitrogen atom. Metal complexes are biologically more active than that of Schiff base. Highest in vitro antibacterial activity observed with Fe-TTS complex against *Enterobacter*, *Staphylococcus aureus* and *Bacillus subtilis* while the Co-TTS complex has the highest activity against *Escherichia coli*. Schiff base and metal complexes are more active towards Gram negative bacterial strains compared to Gram positive bacterial strains.

Supplementary Data

Supplementary data associated with this article are available in the electronic form at

[http://nopr.niscair.res.in/jinfo/ijca/IJCA_60A\(08\)1048-1054_SupplData.pdf](http://nopr.niscair.res.in/jinfo/ijca/IJCA_60A(08)1048-1054_SupplData.pdf).

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