



SYNTHESIS, CHARACTERIZATION AND DNA CLEAVAGE STUDIES OF MONONUCLEAR COBALT (II) COMPLEX DERIVED ISATIN AND ANILINE

Sathya Babu

Department of chemistry, Sri Sarada College for women (Autonomous), salem-16

Akila Ekamparam

Department of chemistry, Sri Sarada College for women (Autonomous), salem-16
Corresponding Author

ABSTRACT The mononuclear Schiff base and its cobalt (II) complex is derived by the condensation of isatin and aniline. The cobalt chloride is added to this ligand in the ratio 1:2 to form mononuclear cobalt complex $[ML_2X_2]$ where M = metal, L = Schiff base ligand, X = chloride ions. The characterization such as elemental analysis, molar conductance, and magnetic moment, IR, UV and 1H NMR for ligand and its complexes have been studied. The spectral data shows that it is in distorted octahedral geometry. The pUC18 DNA cleavage study was monitored by agarose gel electrophoresis method. From this study it was found that the mononuclear Co(II) complex cleaves pUC18 DNA in the presence of the oxidant H_2O_2 . From the antibacterial activity the cobalt(II) complex shows higher activity than the ligand.

KEYWORDS : Schiff base, DNA Cleavage, antibacterial activity

Introduction

Schiff base compound containing an azomethine group which is formed by the condensation of amine with an active carbonyl [1]. Schiff base form complexes with many transition metals that have important application in various fields. Development of a new chemotherapeutic Schiff base is now attracting the attention of medicinal chemist [2]. The azomethine group present in Schiff base ligand is responsible for the biological activities such as antifungal, antibacterial, antiviral, anticancer and herbicidal activities [3-4].

Mono and polynuclear cobalt complexes shows variety of application such as catalysts, electron transfer mediators in dye sensitized solar cells, antiviral agents and molecular nano magnets [5]. Hexamine cobalt complex induce DNA condensation [6]. DNA binding studies and cobalt (II) complex have been investigated for their anticancer properties [7]. Cobalt Schiff base complex as potent bioactive agent has opened up a new avenues for the development of more effective drugs [8].

Particularly, Isatin derived metal complexes shows biological activities such as antiviral, antitumour, antiangiogenic, antifungal, anticancer, antitubercular, neurophysiological, neuropharmacological, anti-HIV and anti-malarial agent. Recently it found application as enzyme inhibitor in the inhibition of cysteine [9-10]. Isatin is a versatile lead molecule for designing bioactive agents and its derivatives [11]. In view of versatile importance of cobalt and isatin derived complex, the present investigation throw light on synthesis, characterization, DNA cleavage, antibacterial activity of Schiff base ligand and its complexes.

Materials and methods

Hydrated cobalt(II) chloride was purchased from Alright and Wilson. Aniline, ethanol, isatin, acetone, dimethyl formamide, chloroform (Nice) were used as received. Elemental analysis were done with CHN analyzer. The IR spectra were recorded on the Perkin Elmer FT-IR 8300 model spectrometer using KBr disc in the region 4000-200 cm^{-1} . Molar conductance of the complex in DMF solution was measured using ELICO CM 185 Conductivity Bridge. UV- Visible spectra were recorded on Double beam spectrophotometer cyber lab between 200-800 nm by using DMF solvent. 1H NMR of a ligand were carried out on a Bruker Spectrometer at 400MHz in DMSO d_6 .

Synthesis of Schiff base ligand

The Schiff base ligand was synthesised by adding isatin (1mM) (0.294g) to an ethanolic solution of aniline (2Mm) (3.72 ml). The mixture was refluxed for 21/2 hrs. Then the solution of the ligand was kept aside for slow evaporation and colored precipitate was collected and dried in air.

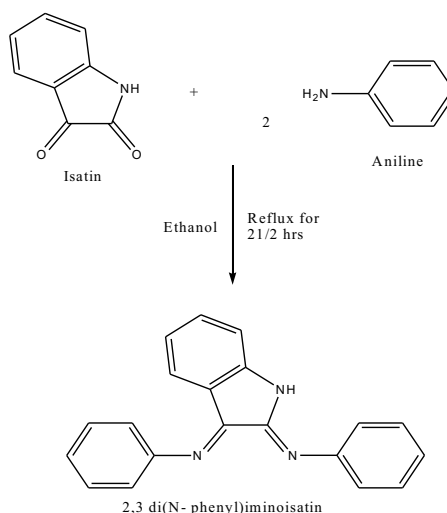


Figure 1. Synthetic methodology of Schiff base

Synthesis of Schiff base cobalt(II) complex

An ethanolic solution of ligand was mixed with cobalt(II) chloride (1Mm) (0.258g) in ethanol solution in the ratio 2:1 with constant stirring. The mixture was then refluxed for 4 1/2 hrs. Then the precipitated complex is filtered off washed with ethanol, dried in vacuo.

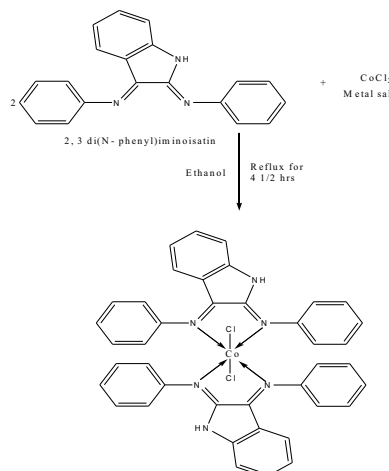


Figure 2. Synthesis of Schiff base cobalt(II) complex

Antibacterial activity

The *in-vitro* antibacterial screening effects of the synthesised ligand

and cobalt (II) complex were tested against four bacterial strains namely *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Klebsiella pneumonia* by disc diffusion method. The solution of complex is made with DMF and the concentrations of 25, 50, 75, 100 $\mu\text{g/ml}$ were prepared. The test solutions were added and petridishes are incubated for 24 hrs. The inhibition zone was developed and it is compared with standard streptomycin.

DNA Cleavage

The cleavage of pUC18 DNA in the presence of H_2O_2 has been studied by gel electrophoresis. The reaction mixture 20 μl contains pUC18 DNA, 50 Mm tris-HCl, pH 7.4, 50mm NaCl, 10Mm H_2O_2 added in a different volume, followed by adding Millipore water for final volume. It was incubated for 1 hr at 37 $^\circ\text{C}$.

Results and discussions

The compound corresponds to the composition ML_2X_2 , where M= Co(II) metal, L= ligand, X= Chloride ion. The analytical data of the synthesised ligand and complex is shown in table-1. It shows that cobalt(II) complex is colored, non hygroscopic, stable in air. It is soluble in DMF, DMSO and partially soluble in common organic solvents such as ethanol, methanol, and chloroform.

Table 1. Analytical data of the Schiff base ligand and its mononuclear cobalt(II) complex

Compound	Molecular Formula	color	Yield %	Melting Point ($^\circ\text{C}$)	% of Nitrogen		% of Metal	
					Cal	Exp	Cal	Exp
L	$\text{C}_{20}\text{H}_{15}\text{N}_3$	Yellow	75	158	14.14	14.12	-	-
CoL_2X_2	$\text{Co}(\text{C}_{40}\text{H}_{30}\text{N}_6\text{Cl}_2)$	Brown	60	>250	11.61	11.59	8.15	8.13

Molar conductance

The molar conductance value of the complexes in 10^{-3} M DMF solution reveals that the complex is nonelectrolytic in nature [12].

IR Spectra

The IR spectra of Schiff base ligand were compared with cobalt(II) complex give information about the coordination site. The important IR bands for the ligand and its metal complex are shown in Table 2. The IR spectrum of the ligand shows a band at 1613.67 cm^{-1} corresponds to $\nu(\text{C}=\text{N})$ which is shifted to a lower frequency in the complex at 1612.10 cm^{-1} which indicates the involvement of $\nu(\text{C}=\text{N})$ nitrogen in coordination to the metal ion [13]. For the synthesised mononuclear Schiff base complex $\nu(\text{C}-\text{C})$ and $\nu(\text{C}=\text{N})$ bands occur at $1268.30 - 1318.50\text{ cm}^{-1}$ and $1613.67 - 1612.10\text{ cm}^{-1}$. The shifting of $\nu(\text{C}-\text{C})$ towards higher frequency as compared to the ligand (1268.30 cm^{-1}) is due to the conversion of hydrogen bonded structure into a covalent metal bonded structure. The bands in the range of 317.50 cm^{-1} and 490.59 cm^{-1} are assignable to $\nu(\text{M}-\text{Cl})$ and $\nu(\text{M}-\text{N})$ respectively are providing additional support to progress the M—L bond through imino nitrogen and chloride ions in the complex [14].

Table 2. Infrared Spectroscopic Data of the Schiff Base Ligand and its mononuclear metal complex

Compounds	$\nu(\text{C}=\text{N})$ (cm^{-1})	$\nu(\text{C}-\text{C})$ (cm^{-1})	$\nu(\text{C}=\text{C})$ (cm^{-1})	$\nu(\text{N}-\text{H})$ (cm^{-1})	$\nu(\text{M}-\text{Cl})$ (cm^{-1})	$\nu(\text{M}-\text{N})$ (cm^{-1})
$\text{C}_{20}\text{H}_{15}\text{N}_3$	1613.67	1268.30	1591.27	3162.40	--	--
$\text{Co}(\text{C}_{40}\text{H}_{30}\text{N}_6\text{Cl}_2)$	1612.10	1318.50	1510.47	3216.16	317.50	490.59

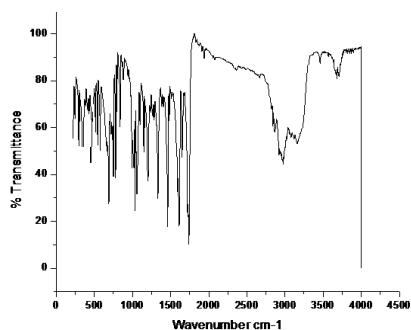


Figure 3. Infrared spectrum of $\text{C}_{20}\text{H}_{15}\text{N}_3$

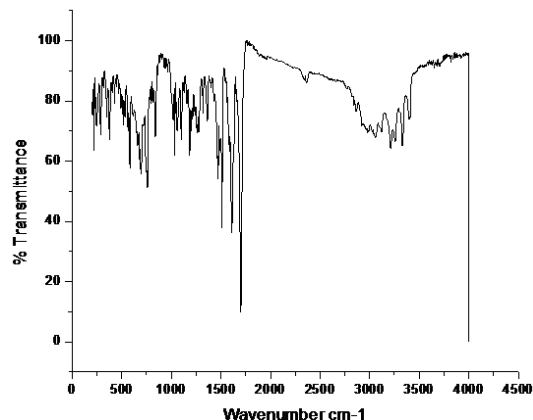


Figure 4. Infrared spectrum of $\text{Co}(\text{C}_{40}\text{H}_{30}\text{N}_6\text{Cl}_2)$

Electronic spectra

The electronic spectra of the free ligand and Co(II) complex are listed in Table-3. The absorption band due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transition is higher for free ligand than Co(II) complex due to the coordination of the ligand with metal ion [15]. The moderately intensive band was observed in the range of 346-379 nm which might be due to $n \rightarrow \pi^*$ transition and strong band was observed in the range of 272- 324nm due to $\pi \rightarrow \pi^*$ transition [16]. The complex shows intense bands in the region of 435 nm which is assigned to be L – M charge transition. The Co(II) complex exhibit three bands in the region of 628, 647, 693 nm is due to ${}^4\text{T}_1\text{g} \rightarrow {}^4\text{T}_2\text{g}(\text{F})$ (ν_1), ${}^4\text{T}_1\text{g} \rightarrow {}^4\text{A}_2\text{g}(\text{F})$ (ν_2) and ${}^4\text{T}_1\text{g} \rightarrow {}^4\text{T}_1\text{g}(\text{P})$ (ν_3) transitions. These shows that Co(II) complex is in distorted octahedral geometry [17].

Table 3. Electronic Spectral data of Schiff base ligand and its Co(II) complex.

Compounds	Absorbance (nm)				Geometry of the complex
	$\pi \rightarrow \pi^*$	$n \rightarrow \pi^*$	L \rightarrow M	d-d	
$\text{C}_{20}\text{H}_{15}\text{N}_3$	324	379	-	-	-
$\text{Co}(\text{C}_{40}\text{H}_{30}\text{N}_6\text{Cl}_2)$	272	346	435	628, 647, 692	distorted Octahedral

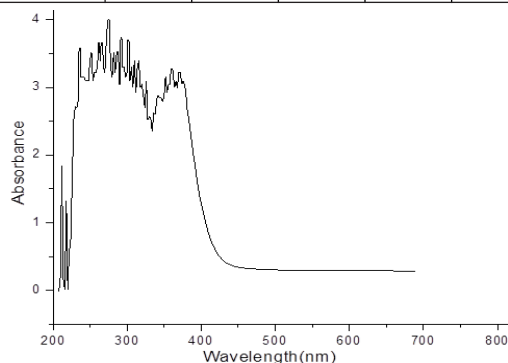


Figure 5. Electronic Spectra of $\text{C}_{20}\text{H}_{15}\text{N}_3$

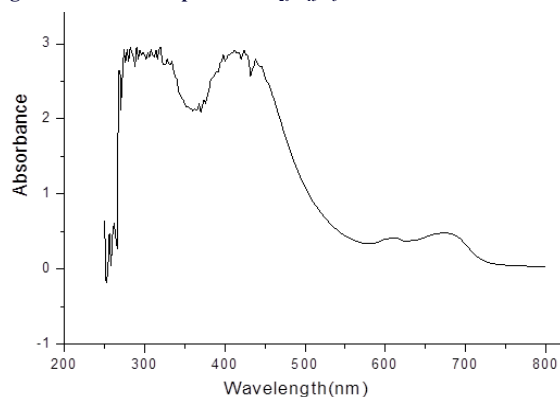


Figure 6. Electronic Spectra of Co(II) complex

¹H NMR Spectra

The ¹H NMR spectrum of the Schiff base ligand was recorded in DMSO d₆. A peak appeared at 8.29 ppm corresponds to azomethine group. A peak at 6.4-7.6 ppm assigned to be aromatic protons [18].

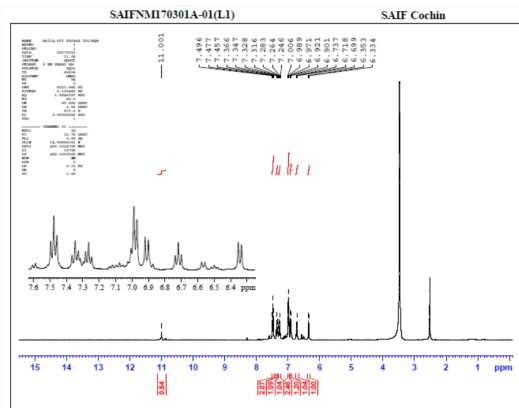


Figure 7. ¹H NMR spectrum of C₂₀H₁₅N₃

Antibacterial activity

The antibacterial activity of Schiff base ligand and its Co(II) complex was tested against two gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and two gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*) using streptomycin as standard. The zone of inhibition of Co(II) complex is higher than the ligand. So complex shows higher activity than the ligand. This is due to chelation theory. The metal present in the complex get adsorbed on the cell wall of bacterial strains which involve in the respiratory process and block the synthesis of protein. Finally it kills the microorganism [19-20].

Table 4. Antibacterial activity for Schiff base ligand and its mononuclear metal complex

Compounds	Zone of inhibition (mm)															
	Gram positive bacteria								Gram negative bacteria							
	<i>Staphylococcus aureus</i>				<i>Bacillus subtilis</i>				<i>Escherichia coli</i>				<i>Klebsiella pneumoniae</i>			
	Concentration (µg/mL)															
	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
C ₂₀ H ₁₅ N ₃	7	7	8	9	7	7	9	9	6	6	7	8	7	8	8	9
Co(C ₄₀ H ₃₀ N ₆ Cl ₂)	10	11	12	14	10	9	12	13	9	9	10	11	10	12	15	17
Streptomycin	16	17	20	22	14	15	17	20	12	13	16	20	13	14	16	18

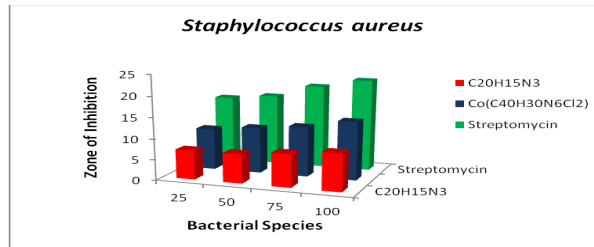


Figure 8. Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complex against *Staphylococcus aureus*

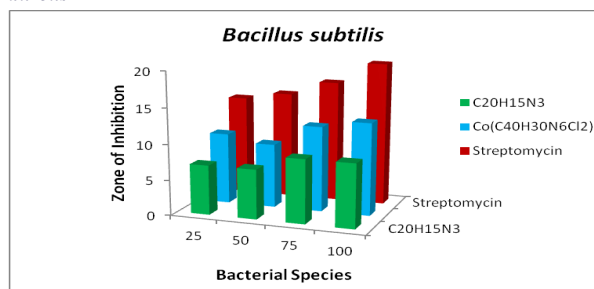


Figure 9. Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complex against *Bacillus subtilis*

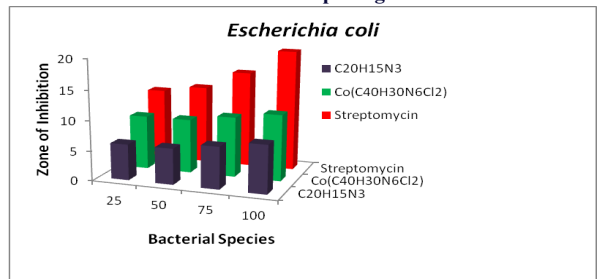


Figure 10. Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complex against *Escherichia coli*

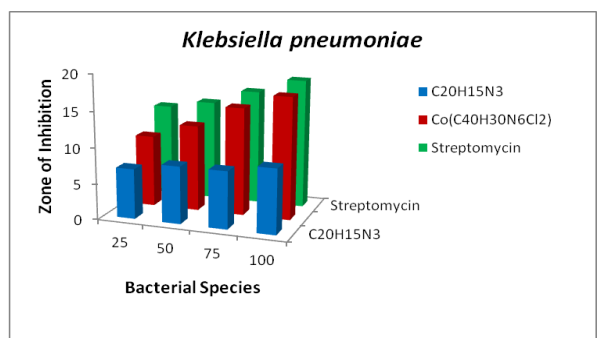


Figure 11. Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complex against *Klebsiella pneumoniae*

DNA Cleavage

DNA plays an important role in life process. Cleavage of DNA is used to treat cancer and some other diseases in medicinal field. The synthesised ligand and Co(II) complex were used for DNA Cleavage activity by agarose gel electrophoresis method. The supercoiled form is cleaved to nicked circular form and it further converted to linear form. The mononuclear Co(II) complex is used to cleave pUC18 DNA induced by H₂O₂. From the observed results, it is concluded that the complex effectively cleave the DNA as compared to ligand [21-22].

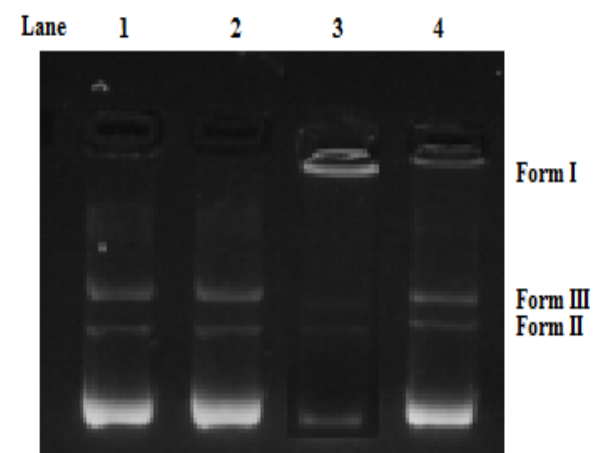


Figure 12. Changes in the agarose gel electrophoretic pattern of pUC18DNA induced by H₂O₂ and synthesised compounds. Lane 1- DNA alone; Lane 2- DNA alone + H₂O₂; Lane 3- DNA + C₂₀H₁₅N₃ + H₂O₂; Lane 4- DNA + Co(C₄₀H₃₀N₆Cl₂)

Conclusion

The present study revealed that the Co(II) complex is in distorted octahedral geometry. The IR spectra provide information about the coordination site of ligand and metal ion. The result of antibacterial activity shows that the Co(II) complex exhibit higher activity than ligand. The DNA cleavage shows that the complex cleave more efficiently than the ligand.

References

1. Sushil Gupta, Peter Hitchcock, Ganpat Argal, *Inorganica chimica acta*, 361 (2008) 2139.
2. Juan Anaconda, Javier santaella, *Spectrochimica acta*, 115 (2013) 800.
3. Bernadette Creaven, Michael Devereux, Agnieszka Foltyn, Siobhan Mc Clean, Georgina Rosair, Venkat Reddy Thangella, Maureen Walsh, *Polyhedron*, 29 (2010) 813.
4. Sachindranath Pal, Anil Kumar Barik, Samik Gupta, Somnath Roy, Tarak Nath Mandal, Arijit Hazra, Mohamed Salah El Fallah, Ray Butcher, Shie Ming Peng, Gene Hsiang Lee, Susanta Kumar Kar, *Polyhedron*, 27 (2008) 357.
5. Michel Fleck, Debasis Karmakar, Mahendra Ghosh, Anupam Ghosh, Rajat Saha, Debasis Bandyopadhyay, *Polyhedron*, 34 (2012) 157.
6. Elif Gungor, Selma Celen, Dilek Azaz, Hulya Kara, *Spectrochimica acta* 94 (2012) 216.
7. Mehdi Saiehi, Raziye Taherlo, *Inorganica chimica acta* 14 (2014) 216.
8. Hasti Iranmanesh, Mahdi Behzad, Giuseppe Bruno, Hadi Amiri Rudbari, Hossein Nazari, Abolfazl Mohammadi, Omid Taheri, *Inorganica chimica acta*, 395 (2013) 81.
9. Akbar Ali, Hih Junaidah Hj Abu Bakar, Mirza, Smith, Guhan, Berhardt, *Polyhedron* 27 (2008) 71.
10. Jaidevi, Nisha Batra, *Spectrochimica acta* 135 (2015) 710.
11. Magdyshebl, *Spectrochimica acta* 117 (2014) 127.
12. Raman, Ravichandran, Thangaraja, *J. Chem. Sci.*, 116 (2004) 215.
13. Nabil Ramadan Bader, *RJC*, 3 (2004) 215.
14. Geetha, Shraavan kumar, Muralidhar Reddy, Ravikrishna, Sarangapani, Krishna Reddy, Ravinder, *Spectrochim. Acta*, 77 (2010) 911.
15. Raman, Jayamurugan, Sakthivel, Mitu, *Spectrochim. Acta*, 75 (2010) 88.
16. E.Q. Gao, W.M. Bu, G.M. Yang, D.Z. Liao, Z.H. Jiang, S.P. Yan and G.L. Wang, *J. Chem. Soc., Dalton Trans.*, 1431, (2000).
17. A.K. Varshney, S. Varshney and H.L. Singh, *Synth. React. Inorg. Met. Org. Chem.*, 29, (1999) 245.
18. Z.H. Wahab, M.M. Mashaly, *Chem. Pap.*, 25 (2005) 59.
19. N. Sengottuvelan, J. Manonmani, M. Kandaswamy, *Polyhedron*, 21 (2002) 2767.
20. N. Raman, S. Sobha, *J. Serb. Chem. Soc.* 75 (2010) 16.
21. K. Dhahagani, S. Mathan Kumar, G. Chakkaravarthi, K. Anitha, J. Rajesh, A. Ramu, G. Rajagopal, *Spectrochimica acta* 117 (2014) 87-94.
22. K. T. Joshi, A. M. Pancholi, K. S. Pandya and A. S. Thakar, *J. Chem. Pharm.* 3 (2011) 741.